Saskatchewan
Agriculture, Food
and Rural
Revitalization

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

20040362

ANTIMICROBIAL RESISTANCE AND ANTIMICROBIAL USE IN WESTERN CANADIAN SWINE

Funded by: The Agriculture Development Fund

August, 2005

Prepared by: University of Saskatchewan

105-07586/

Final Report for the
Saskatchewan Department of Agriculture, Food and Rural Revitalization

The occurrence of antimicrobial resistant *Campylobacter, Salmonella* and *E. coli* enteric bacteria; The use of antimicrobial drugs in western Canadian swine operations; and The associations between antimicrobial use and resistance

Project Number: 20040362
MIDAS: #000341

Cheryl Waldner, DVM, PhD
Leigh Rosengren DVM

Large Animal Clinical Sciences
Western College of Veterinary Medicine
University of Saskatchewan
52 Campus Drive
Saskatoon, SK S7N 5B4

July 11, 2005

ABSTRACT 3

INTRODUCTION 4

Project Objectives 5

General Methods 5
Farm Inclusion Criteria 5
Fecal Sample Collection 5
Data Management and Statistical Methods 6

ANTIMICROBIAL USE 8

Purpose 8

Methods 8

Results 10

Conclusions 21

CAMPYLOBACTER 22

Methods 22
Isolation 22
Antimicrobial Sensitivity Testing – E test 22

Antimicrobial Sensitivity Results 23

Conclusions 27

SALMONELLA 29

Methods 29
Isolation 29
Serotyping 30
Antimicrobial Sensitivity testing – Broth Microdilution 32

Antimicrobial Sensitivity Results 32

Conclusions 37

ESCHERICHIA COLI 38

Methods 38
Isolation 38
Antimicrobial Sensitivity testing – Broth Microdilution 38

Antimicrobial Sensitivity Results 39

Conclusions 43

ASSOCIATIONS BETWEEN ANTIMICROBIAL USE AND RESISTANCE 44

Purpose 44

Methods 44

Results 46
General 46
Tetracycline resistance 47
Sulfamethoxazole resistance 49

Conclusions 50

CONCLUSIONS AND RECOMMENDATIONS 52

ACKNOWLEDGEMENTS 53

LITERATURE CITED 54

Abstract

This study had three primary objectives. The first was to describe the antimicrobial resistance (AMR) in *Campylobacter*, *Salmonella*, and *Escherichia coli (E. coli)* isolates. The second was to quantify the antimicrobial use (AMU) for the previous twelve months on twenty swine farms in western Canada. The final objective was to examine the association between herd level antimicrobial use and *E. coli* antimicrobial resistance. Herds from Saskatchewan and Alberta were sampled in the summer of 2004. A total of 468 *Salmonella*, 1439 *E. coli*, and 405 *Campylobacter* isolates were harvested from fresh fecal samples. *Salmonella* and *E. coli* isolates were tested for antimicrobial sensitivity using broth microdilution (Sensititre®, TREK Diagnostic Systems Inc., Cleveland, Ohio) and the 2002 National Antimicrobial Resistance Monitoring System (NARMS) panel. Antimicrobial sensitivity of *Campylobacter* isolates were preformed with the E-test. (?) The *Salmonella* isolates were most resistant to tetracycline (29%) and sulfamethoxazole (24%) with a total of 26.5% of the isolates being resistant to two or more antimicrobials. The *E. coli* isolates were also most resistant to tetracycline (67%) and sulfamethoxazole (46%) with a total of 57.1% of the isolates being resistant to two or more antimicrobials. *Campylobacter* isolates were most resistant to erythromycin (90%), azithromycin (77%) and clindamycin (73%). Eighty-five (85%) percent of the isolates were resistant to two or more antimicrobials. The highest total incidence of antimicrobial use was reported for nursery pigs. For all phases of production, administration of antimicrobials through feed was the most common route accounting for 88% of antimicrobial treatment incidence. Models found a positive association between antimicrobial treatment incidence and *E. coli* resistance to tetracycline and sulfamethoxazole at the pen level in grow-finish pigs.

Introduction

The development of resistance to antibiotics, by intrinsically sensitive bacteria, is a growing concern in human and veterinary medicine. Antimicrobial resistant bacteria from food animals can impact human health in three fundamental ways. Resistant zoonotic pathogens, such as *Salmonella* and *Campylobacter*, can cause foodborne infections. These infections may be more virulent, leading to increased duration and severity of infection [1]. If antimicrobial treatment is required, options may be limited forcing the use of less effective, more expensive or more toxic alternatives with an increased risk of treatment failure [2, 3]. Secondly, people taking antimicrobials for any reason are at increased risk for foodborne infections with resistant bacterial. Lastly, resistant commensals, such as *Escherichia coli*, may exchange resistance genes with human commensals or pathogens while passing through the human gastrointestinal tract [2, 4]. *Salmonella, Campylobacter* and generic *E. coli* isolates from swine, both in Canada and internationally, have been shown to have high levels of resistance to some antimicrobials [5-8]. Therefore, understanding and monitoring the development of AMR in swine is an important public health activity.

It is widely accepted that antimicrobial use exerts selective pressure on bacteria to develop resistance. Significant relationships between the use of feed antimicrobials and the level of AMR in fecal *E. coli* of market age hogs have been demonstrated [9] indicating that AMU is associated with AMR in swine. Quantitative data on the use of antimicrobials in swine production in western Canada and understanding of the relationship between AMU and AMR is limited.

Monitoring and understanding the development of AMR is a stated priority of numerous national and international agencies, including the World Health Organization, the Health Canada Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health and the National Antimicrobial Resistance Monitoring System of the United States [10-14]. These agencies recommend the monitoring of human zoonotic pathogens, commensal bacteria and animal pathogens [15].

Project Objectives

The objectives of this project were to define the antimicrobial resistance profiles in porcine *Salmonella, E. coli,* and *Campylobacter* from twenty herds in western Canada, to quantify antimicrobial use over the previous twelve months on these same study farms, and to examine the associations between on-farm antimicrobial use and resistance in *E. coli*.

General Methods

Farm Inclusion Criteria

Six clinics (?) with swine veterinarians were contacted from both Saskatchewan and Alberta. Each veterinarian was asked to identify clients with a minimum of 100 sows or 2000 pigs marketed per year. From the lists supplied by eight (?) of these veterinarians, thirteen Saskatchewan and seven Alberta herds were selected. Ten herds were selected for known *Salmonella* status; seven positive and three negative. These were delegated "Phase A" herds and were sampled more intensely for *Salmonella*. The remaining ten herds were not selected based on *Salmonella* status but rather were chosen from as many different veterinarians as possible to maximize the geographical area represented by the sample. Researchers were blind to the treatment status of the herds at the time of selection as information on the amount or type of antimicrobial use was not available. Each herd was visited once between May and September of 2004. Fecal samples and antimicrobial use data were collected and questionnaires were administered to the farm owner or manager.

Fecal Sample Collection

General

Pooled fecal samples were collected from pens selected using a random numbers table. Each pooled fecal sample was composed of five fresh individual fecal samples. The total sample was a minimum of 75 grams. Samples were mixed, divided and shipped overnight to the appropriate laboratory. Each herd was sampled once between May and September of 2004. The following pen-level data were collected; sex, breed, number and age of pigs

in the pen, pen dimensions, floor and wall materials, pen cleanliness and feed type and feeding practices.

Phase A herds

Samples were collected from pigs in all phases of production after weaning including: nursery, grow-finish, and sows. Thirty pooled samples were collected from nursery pigs. Thirty pooled and thirty individual samples, from pigs which also contributed to the pen's pooled sample, were collected from grow-finish pigs. Twenty pooled and ten individual samples from animals not contributing to the pooled sample were collected from sows. All samples were cultured for *Salmonella* by the Agri-Food Laboratories Branch (AFLB), Food Safety Division of Alberta Agriculture, Food and Rural Development, Edmonton, AB. Twenty randomly selected grow-finish pooled samples were cultured for *Escherichia coli* at Prairie Diagnostic Services (PDS), Saskatoon, SK. and twenty or twenty-five were cultured for *Campylobacter* at Laboratory Services Division (LSD), University of Guelph, Guelph, ON.

Phase B herds

Pooled fecal samples were collected from twenty-five pens of grow-finish pigs. All twenty-five samples were cultured for *Salmonella*. The first four farms sampled, representing one hundred fecal samples, were cultured by PDS. Due to a significant cost saving per culture, the remaining six herds (150 cultures) were cultured at LSD. Twenty randomly selected samples per herd were cultured for *E. coli* by PDS. Twenty or twenty-five samples were cultured for *Campylobacter* by LSD.

Data Management and Statistical Methods

Antimicrobial resistance data for all organisms and antimicrobial use data was maintained in a relational database (Microsoft Access, Microsoft Corporation, Redmond, WA, USA). Antimicrobial resistance results were reported as Minimum Inhibitory Concentration (MIC) values. These data were categorized as sensitive or resistant, with intermediate results categorized as sensitive for this report.

Descriptive statistics were preformed with commercially available software (SPSS, ver. 12.0, Chicago, IL, USA and Microsoft Excel, Microsoft Corporation, Redmond, WA, USA). Subject-specific antimicrobial resistance prevalence estimates adjusted for clustering at the herd level were calculated using Restricted Iterative Generalized Least Squares (RIGLES), 2nd order penalized quasi likelihood (PQL) mixed models with a binomial distribution and logit link function (MLwiN ver. 2.0, Center for Multilevel Modeling, Institute of Education, University of London, London, England).

Antimicrobial use

Purpose

This aspect of the project quantified antimicrobial use on study farms. A treatment incidence for antimicrobials administered by feed, water or injection in the twelve months prior to sample collection was calculated for all stages of production; suckling, nursery, grow-finish and sows.

Methods

All inventory and antimicrobial use data were at a herd level with no individual or pen level identification. For the purposes of this study, the term antimicrobial included sulfonamides.

Feed and water antimicrobial use was classified as a group treatment. Data were collected on the phase of production, product or generic equivalent used, number of pigs treated, duration, and dose administered. The reason for use was categorized as growth promotion, disease prevention or disease treatment according to the producer's purpose rather than the dose or duration administered. Treatment incidence per 1000 pig-days was the standardized treatment measure for each antimicrobial use. The formula used was calculated as follows:

$$\frac{\text{(Number of pigs treated) * (duration of treatment in days) * 1000}}{\text{[(Number into phase) + (Number exiting phase) / 2]* (average duration in phase in days)}}$$

The numerator was calculated as the number of pigs exposed multiplied by the duration of treatment. Each day of treatment was considered a unique exposure. The population at risk for each herd and phase was an approximation as all farms had open populations (in the sense that animals entered and left the study) and inventory data were at the herd level. The number of pigs at risk was the average of the number moved in and out of each phase over the last twelve months. This assumed mortality and exits occurred half way

through each phase. The days at risk was the average number of days spent in each phase of production. For herds which sold breeding stock or isowean piglets, a weighted average was calculated to account for these animals.

Products containing more than one antimicrobial were used in feed and water medications. When examining treatment incidence by family or product, combination products were divided into the constituent active ingredients. For all other calculations, combination products were considered one treatment.

All herds provided individual pig antimicrobial use data, either through existing records or completion of a survey. Surveys estimated the treatment incidence per pig day by phase, the most commonly used antimicrobials, typical dose, duration and reason for use. For herds which maintained individual animal treatment records, all records were entered into a Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheet to the level of detail recorded on that farm. The detail recorded varied between herds as existing records were maintained for purposes other than this study.

Individual treatment incidence for a farm and phase was the summation of each individual treatment incidence. Each treatment was categorized by family of antibiotic and active ingredient. Treatment incidence per 1000 pig days was the standardized measurement and used the following formula:

$$\frac{\text{(Number of initial pigs treated)} * 1000}{[\text{(Number into phase)} + \text{(Number exiting phase)}/2] * \text{(time at risk)} * \%\ \text{records available}}$$

Treatments of the same product given to a pig within five days were not considered unique due to insufficient data on treatment duration. Incidence was adjusted for less than twelve months of data (in some herds in some classes) by multiplying the denominator by the percent of records available.

Total treatment incidence for a phase and farm was tabulated as the sum of the feed, water and injectable treatment incidence. As a pig could potentially be treated once by

each route on any given day, the maximum value per phase and farm was 3000 treatments per 1000 pig days.

Results

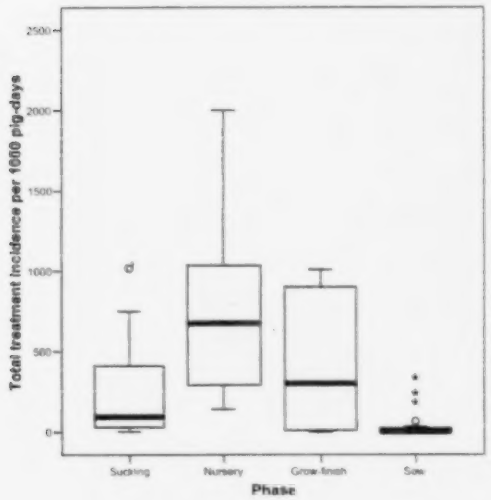
Herd Description

All herds were closed to incoming animals except breeding stock and seven of the twenty herds sold breeding stock. Sixteen herds were located on one site while four were located on more than one site. All herds were farrow-to-finish with a median of 456 sows and an inter-quartile range of 274 to 1042. Based on 2004 Statistics Canada numbers, the herds sampled represented 17.4% of sows farrowed and bred to farrow in Saskatchewan and 3.7% in Alberta [16]. Pigs were classified by the following phases of production; suckling, nursery, grow-finish and sow. On average pigs were weaned at 21 days (s=1.75) at 6.5 kg (s=1.02). They spent 46.4 days (s=15.65) in the nursery and weighed 27.3 kg (s=6.94) at transfer. On average pigs were marketed after 112.3 days (s=27.14) in the grow-finish barn weighing 113.5 kg (s=6.60).

Total antimicrobial use

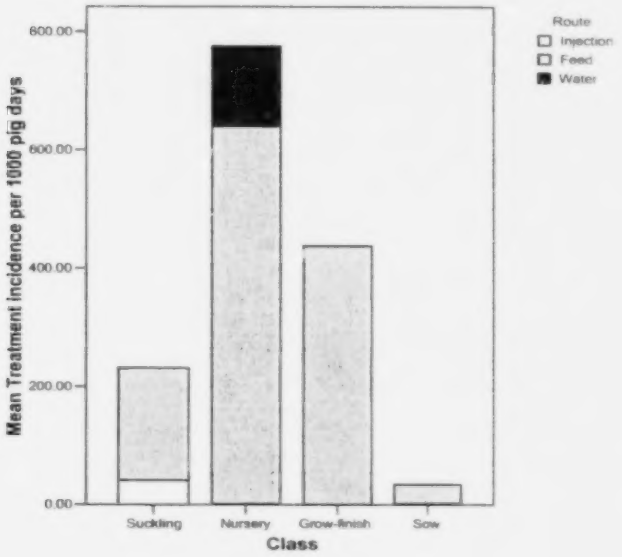
The distribution of total antimicrobial use for all routes by phase is shown in Figure 1. Nursery pigs demonstrated the highest total treatment incidence with a median value of 677 treatments per 1000 pig-days and an IQR of 260 to 1054. Grow-finish pigs had the second highest treatment incidence with a median of 302 and an IQR of 6 to 927. Suckling pigs had a median treatment incidence of 95 with an IQR of 27 to 422. Sows had the lowest treatment incidence with a median of 1.2 and an IQR of 0.3 to 30.2.

Figure 1. Total antimicrobial use for all routes by phase of production



Route of treatment by phase is displayed in Figure 2. Overall, 88.0% of treatment incidence was through feed, 9.1% through water and 2.9% through individual injection. By phase, suckling piglets received 83.2% of medication incidence by feed, 0% by water and 16.8% by injection. Nursery pigs received 82.4% of treatment incidence by feed, 17.4% by water and 0.1% by injection. Grow-finish pigs received 99.7% of treatment incidence by feed, 0.2% by water and 0.1% by injection. Sows received 95.8% of treatment incidence by feed, 0.7% by water and 3.4% by injection. From these results it is evident that water medication is an important route for delivery in nursery animals but not in the other phases. Injectable medication is a relatively common route to treat suckling piglets and sows but not nursery or grow-finish animals.

Figure 2. Mean antimicrobial use by phase and proportion of use by route



Feed Antimicrobial Use

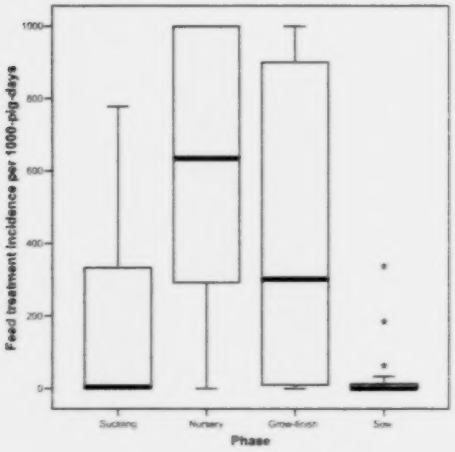
The pattern of feed medication use by farm is shown in Table 1. Feed medication was used in 19 of the 20 herds. All herds that used feed medication also medicated nursery rations. All herds medicating sow rations also medicated grow-finish rations and all herds medicating grow-finish rations also medicated nursery rations. Creep treatment incidence negatively correlated with weaning age (Pearson correlation r = -0.58; $p = 0.007$). Total incidence of feed antimicrobial administration was not correlated with herd size (r=0.15; p=0.19) nor was feed treatment incidence correlated with herd size when examined by phase of production.

Table 1. Pattern of feed antimicrobial use within herds across phases

Herds	Suckling	Nursery	Grow-finish	Sows
1				
1		•		
2	•	•		
5		•	•	
4	•	•	•	
3		•	•	•
3	•	•	•	•

The distribution of feed treatment incidence by phase is shown in Figure 3. Nursery pigs received the highest treatment incidence followed by grow-finish pigs. The median nursery incidence was 634 treatments per 1000 pig-days with seven herds reporting a treatment incidence of 1000 per 1000 pig-days. Grow-finish pigs had a median treatment incidence of 302 treatments per 1000 pig-days with an inter-quartile range of 5 to 927. Creep rations had a median treatment incidence of 3.3 with an inter-quartile range from 0 to 333. Ten herds used no feed medication in creep rations. Sow median treatment incidence was zero; thirteen herds did not use medicated feed. For sows, the 75th percentile was 15 treatments per 1000 pig days.

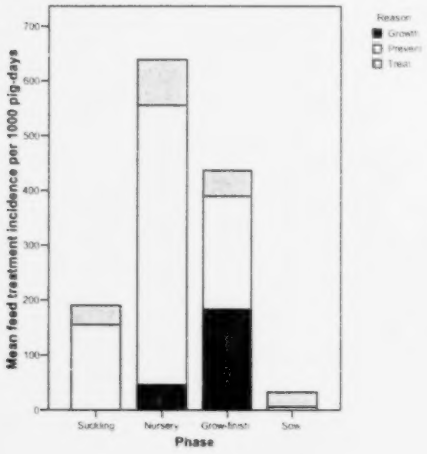
Figure 3. Distribution of antimicrobial treatment incidence per 1000 pig-days via feed by phase of production



Each treatment was categorized by the producer according to the reason for adding medication to the ration (Figure 4). By proportion of treatment incidence, suckling piglets received no feed medication for growth promotion, 81.5% to prevent disease and 18.5% to treat disease. Nursery pigs received 7.2% of their treatment incidence to promote growth, 79.8% to prevent disease and 13.0% to treat disease. Grow-finish pigs received 42.0% of the feed treatment incidence to promote growth, 47.2% to prevent disease and 10.8% to treat disease. Sows received 19.4% of feed treatment incidence to prevent

disease and 80.6% to treat disease. These results demonstrate that growth promotional use of antimicrobials is common only in grow-finish pigs with minor use in nursery pigs. Disease prevention was the overall most common reason for using feed antimicrobials.

Figure 4. Mean feed antimicrobial treatment incidence by phase and reason for use



Feed medication treatment incidence was stratified by herd, phase and product. The mean treatment incidence per 1000 pig-days across the 20 study herds and the mean treatment incidence per 1000 pig-days for herds using a product were calculated (Table 2). In suckling piglets, lincomycin and spectinomycin were the most frequently used product with a mean treatment incidence of 102 treatments per 1000 pig-days respectively. Oxytetracycline was the second most frequently used product (85 treatments per 1000 pig-days) followed by tiamulin (45 treatments per 1000 pig-days). In nursery pigs, the same pattern of product frequency, excluding spectinomycin, was seen however the treatment incidence was consistently more than twice as high as for suckling piglets. In grow-finish pigs, tylosin was the most commonly used product with a mean treatment incidence of 277 treatments per 1000 pig-days followed by lincomycin and chlortetracycline. Tylosin was also the most frequently used product in sows with a mean treatment incidence of 15 treatments per 1000 pig-days. Oxytetracycline and chlortetracycline were the second and third most frequently used products respectively.

Table 2. Mean treatment incidence per 1000 pig-days across all herds and across herds using a product stratified by phase of production

		All herds		Herds using product		
Phase	Product	Mean	St dev	# herds	Mean	St dev
Suckling	Penicillin	41	102	3	270	73
	Sulfonamides	41	102	3	279	73
	Chlortetracycline	85	163	5	341	127
	Oxytetracycline	0	0	0	0	0
	Lincomycin	102	271	3	678	334
	Neomycin	0	0	0	0	0
	Spectinomycin	102	271	3	678	334
	Tiamulin	45	130	4	226	231
	Tilmicosin	0	0	0	0	0
	Tylosin	14	64	1	286	-
Nursery	Penicillin	35	74	4	174	51
	Sulfonamides	35	74	4	174	51
	Chlortetracycline	238	279	13	266	269
	Oxytetracycline	9	39	1	173	-
	Lincomycin	250	332	10	500	320
	Neomycin	9	39	1	173	-
	Spectinomycin	112	238	6	373	316
	Tiamulin	150	227	9	333	232
	Tilmicosin	10	45	1	200	-
	Tylosin	117	252	5	466	315
Grow-finish	Penicillin	1	5	1	24	-
	Sulfonamides	1	5	1	24	-
	Chlortetracycline	36	161	1	720	-
	Oxytetracycline	0	0	0	0	-
	Lincomycin	157	337	5	626	416
	Neomycin	0	0	0	0	-
	Spectinomycin	0	0	0	0	-
	Tiamulin	0	0	0	0	-
	Tilmicosin	3	12	1	52	-
	Tylosin	277	392	11	504	408
Sow	Penicillin	3	14	1	64	-
	Sulfonamides	0	0	0	0	-
	Chlortetracycline	10	41	2	99	122
	Oxytetracycline	14	52	3	95	200
	Lincomycin	2	8	2	18	23
	Neomycin	0	0	0	0	-
	Spectinomycin	0	0	0	0	-
	Tiamulin	0	0	0	0	-
	Tilmicosin	0	0	0	0	-
	Tylosin	15	67	1	300	-

Water Antimicrobial Use

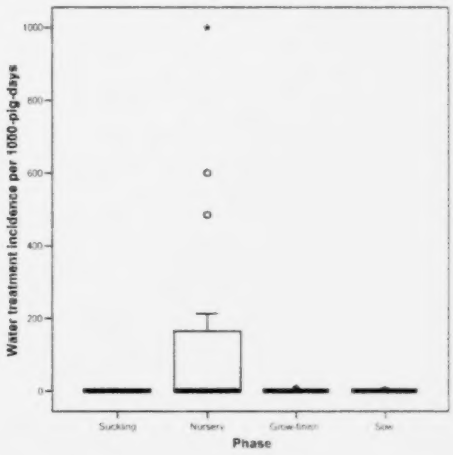
Antimicrobials were administered via water on ten of the study farms. Six herds reported using water medication only in nursery animals, one herd only in grow-finish animals and two herds in both nursery and grow-finish animals (Table 3). One herd owner used water medication in sows only.

Table 3. Pattern of water medication use within herds by phase of production

Herds	Suckling	Nursery	Grow-finish	Sows
10				
6		•		
1			•	
2		•	•	
1				•

The median treatment incidence for water medication was zero for all phases with the distribution shown in Figure 5. The median treatment incidence *of the eight herds using medication* in nursery pigs was 200 with a minimum of 12 and a maximum of 1000. Of the three herds using water medication in grow-finish pigs the lowest treatment incidence was 1 treatment per 1000 pig-days and the highest was 11. The one herd owner using water medication in sows had a treatment incidence of 7 treatments per 1000 pig-days.

Figure 5. Water antimicrobial treatment incidence by phase of production



Water medication was used to prevent disease 86.4% of the time in nursery pigs. The remaining 13.6% of treatment incidence in nursery pigs and all use in grow-finish pigs and sows was to treat disease (Table 4).

Table 4. Reason for use of water antimicrobials by phase of production

Route	Class	Herds	Disease Prevention (%)	Disease Treatment (%)
Water	Nursery	8	86.4	13.6
	Grow-Finish	3	0	100
	Sows	1	0	100

Use of water medication was stratified by herd, phase and product used. The mean treatment incidence by product is demonstrated across the 20 herds in the study and across the herds reporting use of that product (Table 5). Data are not presented for sows as only one herd used water medication. In both nursery and grow-finish pigs the most frequently used product was penicillin G potassium with a mean treatment incidence of 85 treatments per 1000 pig-days and less than 1 treatment per 1000 pig-days respectively across all study herds. The four producers reporting penicillin G potassium use in nursery pigs had a mean treatment incidence of 423 treatments per 1000 pig days. The two producers reporting penicillin G potassium use in grow-finish animals had a mean treatment incidence of 6 treatments per 1000 pig-days.

Table 5. Mean treatment incidence per 1000 pig-days by active ingredient for antimicrobials administered through the water for all study herds and for herds using product

		All herds		Herds using product		
Phase	Product	Mean	St dev	#herds	Mean	St dev
Nursery	Penicillin	85	232	4	423	387
	Amoxicillin	10	43	1	194	-
	Sulfonamide	11	35	2	108	50
	Tetracycline HCl	30	134	1	600	-
	Neomycin	34	134	3	228	324
Grow-finish	Penicillin	0.6	2	2	6	7
	Amoxicillin	0	0	0	0	-
	Sulfonamide	0	0	0	0	-
	Tetracycline HCl	0.3	1	1	5	-
	Neomycin	0	0	0	0	-

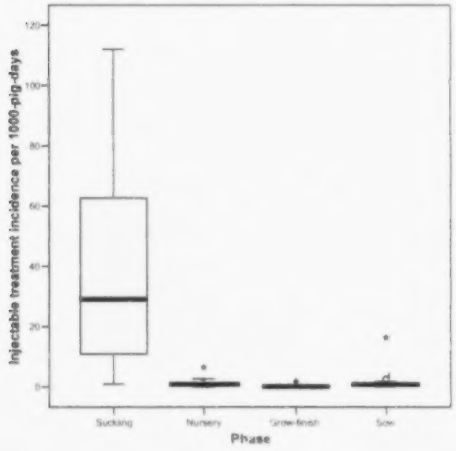
Individual Antimicrobial Use

Individual antimicrobial use data came from two sources; existing records and surveys. For herds which maintained individual records, the level of detail maintained varied. All herds recorded the phase of production, number of pigs treated and product used. The recording of dose and duration of treatment was variable as was the phases for which records were maintained. One of the 20 herds maintained individual treatment records on suckling piglets, four on nursery pigs, fourteen herds on grow-finish animals and twelve on sows. Five herds were not able to provide existing records for any class of production. Not all herds were able to provide a complete twelve months of records for all phases. Median treatment incidences are displayed in Table 6 by phase and source of data. Nine herds routinely medicated all suckling piglets and one herd routinely treated all sows post-farrowing to prevent disease. All other reported use was for treatment purposes. Suckling piglets received the highest treatment incidence with a median of 29.1 treatments per 1000 pig-days and an IQR of 11.5 to 57.1. Nursery pigs received the second highest treatment incidence followed by sows. Grow-finish pigs received the lowest individual animal treatment incidence. The distribution of treatment incidences, with sources combined is shown in Figure 6.

Table 6. Individual treatment incidence per 1000 pig-days by phase and source of data

Class	Records	Herds	Median	Lower IQR	Upper IQR
Suckling	All	20	29.1	11.5	57.1
	Individual	1	9.9	-	-
	Survey	19	30.9	15.2	62.7
Nursery	All	19	0.89	0.34	1.5
	Individual	4	0.34	0.12	1.2
	Survey	15	0.94	0.38	1.8
Grow-Finish	All	19	0.12	0.02	0.37
	Individual	14	0.25	0.06	0.54
	Survey	5	0.00	0.00	0.00
Sow	All	20	0.75	0.33	1.1
	Individual	12	0.92	0.26	1.1
	Survey	8	0.67	0.33	2.0

Figure 6. Distribution of individual animal treatments by phase



The frequency of use and average treatment incidence by product is shown in Table 7. Only data from individual records was used to create this table. Suckling piglet data are not presented as existing records were available from only one herd. Short acting penicillin was the most frequently used product in all phases of production with a median treatment incidence ranging from 0.03 treatments per 1000 pig-days in grow-finishers to 0.15 treatments per 1000 pig-days in nursery pigs. Trimethoprim-sulfadoxine was

consistently within the top three most frequently used products in all phases of production and was the only combination product reported.

Table 7. Frequency of use and treatment incidence per 1000 pig-days by active ingredient for antimicrobials administered by injection based on herds providing individual records

Phase	Product	Herds	Median	Inter-quartile range	
				Lower	Upper
Nursery	Ampicillin	4	0.00	0.00	0.00
	Ceftiofur	4	0.05	0.00	0.19
	Short Acting penicillin	4	0.15	0.02	0.34
	Long Acting penicillin	4	0.00	0.00	0.22
	Trimethoprim-sulfadoxine	4	0.05	0.01	0.45
	Short acting oxytetracycline	4	0.00	0.00	0.09
	Long acting oxytetracycline	4	0.03	0.01	0.09
	Lincomycin	4	0.00	0.00	0.03
	Tiamulin	4	0.00	0.00	0.00
	Tylosin	4	0.00	0.00	0.00
Grow-finish	Ampicillin	14	0.00	0.00	0.00
	Ceftiofur	14	0.00	0.00	0.01
	Short Acting penicillin	14	0.03	0.01	0.25
	Long Acting penicillin	14	0.00	0.00	0.02
	Trimethoprim-sulfadoxine	14	0.01	0.00	0.03
	Short acting oxytetracycline	14	0.00	0.00	0.00
	Long acting oxytetracycline	14	0.00	0.00	0.02
	Lincomycin	14	0.00	0.00	0.01
	Tiamulin	14	0.00	0.00	0.00
	Tylosin	14	0.00	0.00	0.00
Sow	Ampicillin	12	0.00	0.00	0.00
	Ceftiofur	12	0.00	0.00	0.00
	Short Acting penicillin	12	0.12	0.06	0.42
	Long Acting penicillin	12	0.00	0.00	0.03
	Trimethoprim-sulfadoxine	12	0.05	0.00	0.13
	Short acting oxytetracycline	12	0.00	0.00	0.01
	Long acting oxytetracycline	12	0.00	0.00	0.28
	Lincomycin	12	0.00	0.00	0.00
	Tiamulin	12	0.00	0.00	0.00
	Tylosin	12	0.00	0.00	0.02

Conclusions

The frequency of antimicrobial use was highest in nursery and grow-finish pigs. Group treatment incidence, particularly via feed, overshadowed individual pig treatments in all phases of production. Feed medication was the most common route of administration for all phases of production and was used on 95% of study herds. This is higher than the previously reported 88% of herds in Ontario and the United States [17, 18]. The decreasing use of feed medication with pig age is also consistent with these reports. Eighty-eight percent of all treatment incidence occurred via feed. Of this, 16% was reportedly for growth promotion, 69% for disease prevention and 16% for disease treatment. Others have reported similarly low proportions of use for disease treatment although differences in definitions prevent direct comparisons between studies [17-19].

Campylobacter

Methods

Isolation

Laboratory Services Division – University of Guelph – Guelph, ON

Campylobacter culture was preformed at Laboratory Services Division (LSD) using a method originally developed for food specimens. Samples were both direct plated onto Charcoal Selective Media (CSM) and pre-enriched in Rosef's enrichment broth. Rosef's enrichment broth contained 10% FBP solution (1 g each of ferrous sulphate.7H20, sodium metabisulfite and sodium pyruvate in a 10% aqueous solution, equal volumes), VTP (vancomycin 0.5 g, trimethoprim 0.25 g, polymyxin B sulfate 0.035 g and 1 L distilled water), cefoperazone, and cycloheximide. The enriched broth was cultured onto Mueller-Hinton Blood Agar (MHBA) containing the same antibiotics. *Campylobacter jejuni* was used as a positive control and *Escherichia coli* ATCC 25922 was used as a negative control. All samples were incubated microaerophilically in 5% 0_2, 10% CO_2 and 85% N_2.

Typical colonies were selected for darkfield microscopy. Those with a typical corkscrew motility were subcultured onto MHBA for purity. Isolates were presumed *Campylobacter* positive if gram negative with a spiral S-shaped or gull-wing morphology, catalase positive and oxidase positive. One isolate per sample was frozen in glycerol peptone at minus 80°C as well as in beads. Identification of the *Campylobacter* species was done through indoxyl acetate and hippurate hydrolysis.

Antimicrobial Sensitivity Testing – E test

Campylobacter isolates were grown up overnight on a plate of Mueller Hinton Agar and 5% citrated sheep's blood (MHCSGA). Isolates from this culture were emulsified in 2 ml of Mueller Hinton broth to a 0.04 turbidity reading on a Dade Microscan Meter. The inoculated suspension was streaked onto Mueller Hinton Agar + 5% Laked Horse Blood (MHLHBA) plates. Two E test strips were applied per plate and incubated at 37°C for 48

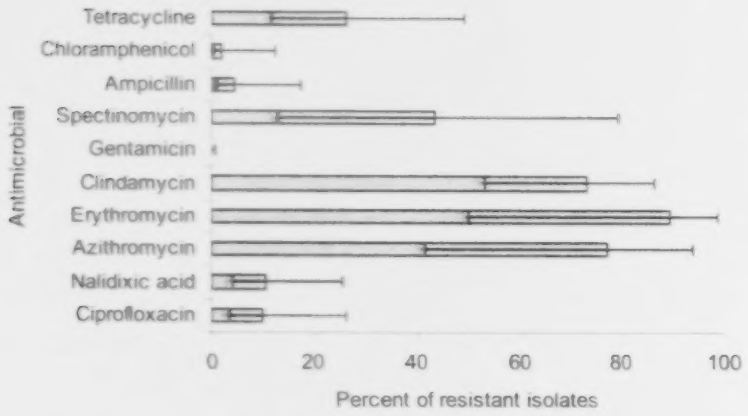
hours or until a *C. coli* control showed significant growth. Bactericidal antibiotic test strips were read at the point where 100% inhibition intersected with the strip. If this occurred between marks on the strip the value was rounded up. Bacteriostatic antimicrobial MIC values were read at 80% inhibition / 20% growth. If this occurred between markings on the strip the number was rounded down to the next marking. Quality control organisms, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Campylobacter jejuni* ATCC 33560 and *Campylobacter coli* ATCC 33559 were run with each set of organisms processed and compared to NCCLS standard. Sensitivity to ten antimicrobials was examined; the range of concentrations tested is shown in Figure 9.

Antimicrobial Sensitivity Results

A total of 444 samples were cultured for *Campylobacter* resulting in 438 positive cultures. On average, 22.2 samples were cultured per herd (range; 20 to 28) with an average herd prevalence of 98.9% (range; 80.8 to 100%). Of the 438 positive cultures, 45 had growth which was too weak to allow for the species to be determined resulting in 393 speciated isolates. Ninety-six percent (379/393) of isolates were *C. coli*, 2.5% were *C. jejuni* and 1.0% *C. lari*. Of the 45 positive cultures from which the species could not be determined, 33 had insufficient growth to allow for antimicrobial sensitivity testing. Therefore, a total of 405 isolates (average; 20.3 per herd) were tested for antimicrobial sensitivity.

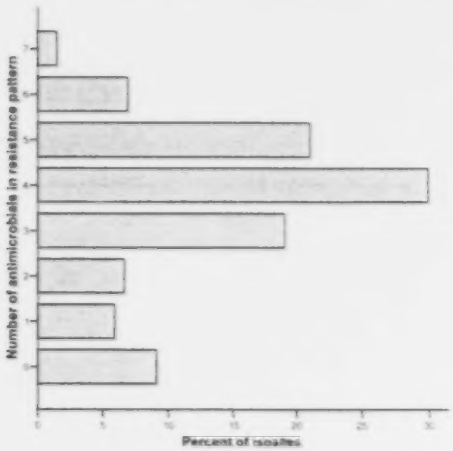
Resistance was noted to all antimicrobials examined with the exception of gentamicin. After adjusting for herd level clustering, the highest frequency of resistance was to the macrolides erythromycin (89.5%), azithromycin (77.0%), and the lincosamide clindamycin (73.1%). Forty-three percent of the isolates were resistant to spectinomycin and 26.5% were resistant to tetracycline. The fluoroquinolones ciprofloxacin and nalidixic acid demonstrated 10.1% and 10.7% resistance respectively. The lowest frequency of resistance was to ampicillin (4.3%) and chloramphenicol (1.9%). (Figure 7)

Figure 7. Prevalence of resistance and 95% confidence intervals for *Campylobacter* isolates adjusted for herd level clustering



Thirty-seven (9.1%) of the isolates were susceptible to all antimicrobials tested, 5.9% resistant to one antimicrobial and 84.9% were resistant to two or more antimicrobials. The most common multiple drug resistance was to four antimicrobials, which was seen in 121 (29.9%) of isolates (Figure 8). Six isolates (1.5%) demonstrated resistance to 7 antimicrobials.

Figure 8. Frequency of multiple antimicrobials in resistance pattern



Thirty-nine different resistance patterns were found in the 405 isolates. The most common was to azithromycin, clindamycin, and erythromycin which occurred in 70 (17.3%) isolates. Resistance to these three antimicrobials with additional antimicrobials was found in 227 (56%) isolates; only 20 isolates with resistance to three or more antimicrobials did not demonstrate resistance to all macrolides (lincosamides) (Table 8).

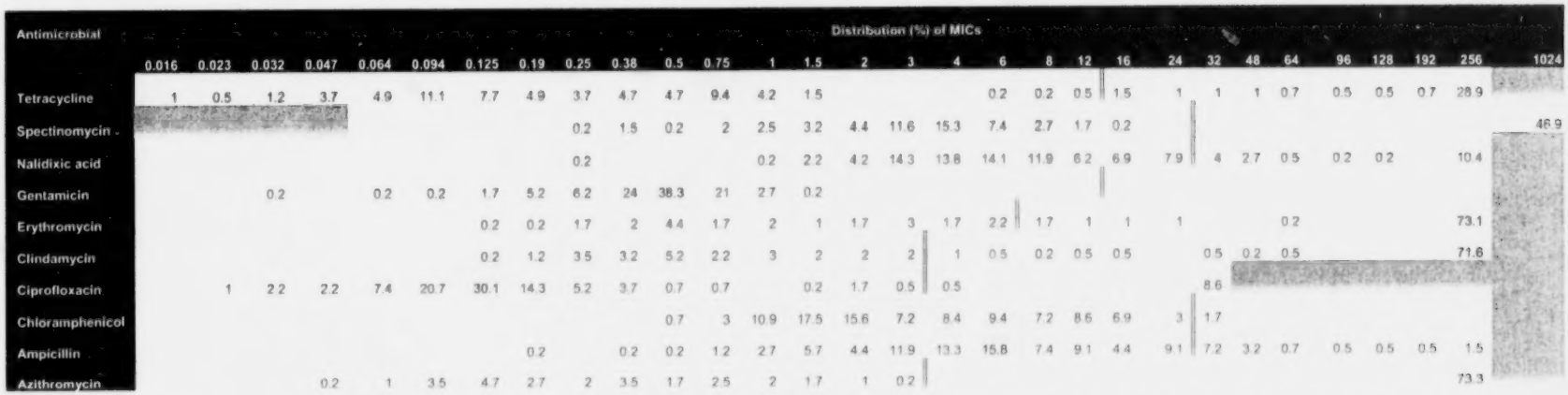
Table 8. Multiple drug resistance patterns

Pattern [a]	Frequency	%	Pattern [a]	Frequency	%
AZ-CM-EM	70	17.3	CM-EM-SC-TC-NA-CL	2	0.5
AZ-CM-EM-SC	60	14.8	CM-SC-TC-NA-CL	2	0.5
AZ-CM-EM-TC	51	12.6	SC-AM	2	0.5
AZ-CM-EM-SC-TC	46	11.4	AZ-CM-EM-CI-NA-AM	1	0.2
No Resistance	37	9.1	AZ-CM-EM-TC-NA	1	0.2
AZ-CM-EM-SC-AM	18	4.4	CM-EM-SC-TC-NA	1	0.2
TC	15	3.7	CM-SC-TC-NA-AM	1	0.2
AZ-CM-EM-SC-CI-NA	13	3.2	SC-TC-NA-AM	1	0.2
CI-NA	13	3.2	CM-EM-TC-AM	1	0.2
AZ-CM-EM-SC-NA	11	2.7	EM-SC-TC-CL	1	0.2
AZ-CM-EM-SC-NA-AM	8	2.0	EM-SC-NA-CL	1	0.2
EM	8	2.0	CM-EM-SC	1	0.2
AZ-CM-EM-SC-CI-NA-AM	6	1.5	SC-TC-NA	1	0.2
AZ-CM-EM-TC-AM	5	1.2	TC-AM	1	0.2
SC-TC-AM	5	1.2	EM-CI	1	0.2
AZ-CM-EM-SC-TC-AM	4	1.0	SC-CL	1	0.2
NA-AM	4	1.0	CM-SC	1	0.2
AZ-CM-EM-NA	3	0.7	TC-NA	1	0.2
EM-TC-CI-NA	3	0.7	SC	1	0.2
SC-TC	3	0.7			

[a] AM: ampicillin; AZ: azithromycin; CI: ciprofloxacin; CL: chloramphenicol; CM: clindamycin; EM: erythromycin; NA: nalidixic acid; SC: spectinomycin; TC: tetracycline

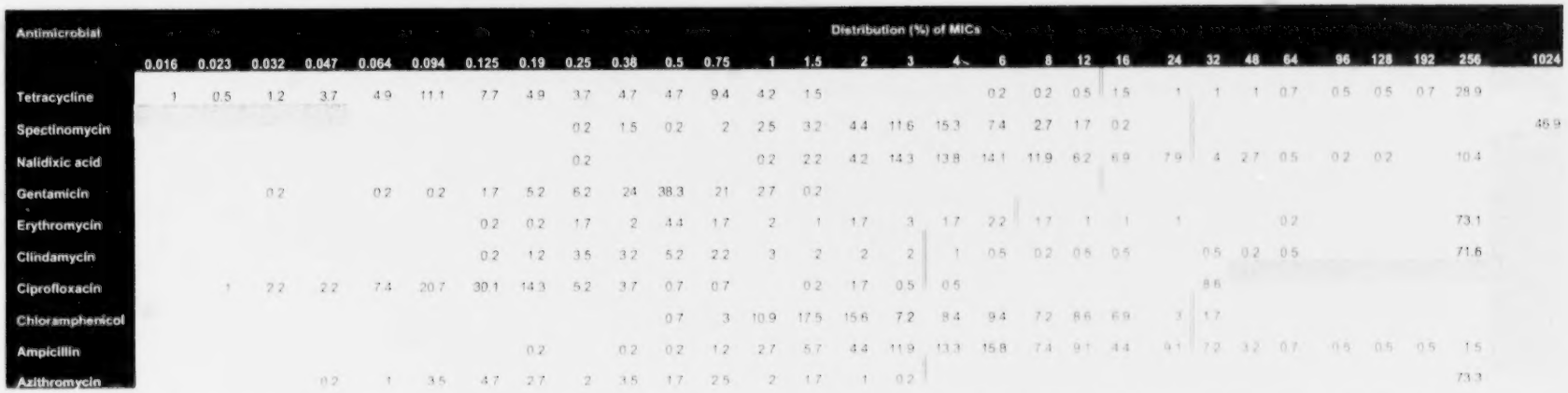
The distributions of the minimum inhibitory concentrations are provided in Figure 9.

Figure 9. Distribution of Minimum Inhibitory Concentration values (percent of isolates)



Note: white area indicates range of antimicrobial concentration tested, light grey number marks the median, vertical double lines mark the breakpoint between intermediate and resistant concentrations

Figure 9. Distribution of Minimum Inhibitory Concentration values (percent of isolates)



Note: white area indicates range of antimicrobial concentration tested, light grey number marks the median, vertical double lines mark the breakpoint between intermediate and resistant concentrations

Conclusions

The prevalence of *Campylobacter* isolated was as high, or higher than, other reports [20]. This was most likely due to each sample consisting of feces from five animals as opposed to one. The predominant species isolated was *Campylobacter coli* which has been commonly reported by others [20-22].

Prior to 2002 standardized antimicrobial sensitivity testing methods for *Campylobacter* had not been released by the NCCLS and to date *Campylobacter* specific sensitive / resistant breakpoints have not been established. Due to this, numerous techniques and cut off values have been used in published literature making the direct comparison of results between studies difficult. This project elected to use the E test for antimicrobial sensitivity to allow for flexibility in the antimicrobial selection and based on the recommendation of the laboratory involved in the project. With the exception of azithromycin, breakpoints utilized by CIPARS and NARMS were used in this project to enable comparison between project results. NARMS and CIPARS classify *Campylobacter* as resistant to azithromycin when the MIC is >2 μg/ml. On the recommendation of laboratory personnel, and based on the population distribution of the isolates in this study, a breakpoint of >4 μg/ml was employed. This is currently under review and may be altered prior to publication of this data, should this happen one isolate which is currently classified as susceptible would be considered resistant.

The isolates in this study were commonly resistant to antimicrobials in the macrolide (azithromycin and erythromycin) and the closely related lincosamide families (clindamycin). High levels of resistance to these antimicrobial families have been reported in Belgium, Italy, Ireland, Denmark, Spain and France in *Campylobacter coli* obtained from pigs at slaughter [20-25]. The high frequency of resistance appears unique to *C. coli* isolates from swine and debate exists in the literature as to whether this resistance is due to frequent macrolide use in swine or an elevated intrinsic resistance in this population of bacteria [22].

Although campylobacteriosis is usually self-limiting, when necessary fluoroquinolones and macrolides are the treatments of choice [26, 27]. The prevalence of resistance to the fluoroquinolones ciprofloxacin and nalidixic acid was 10 and 11% respectively. *C. coli* has had reported resistance to ciprofloxacin ranging from 28 – 100% [20, 25] and from 17 – 100% to nalidixic acid [20, 22] with cross resistance between these antimicrobials being commonly reported. The relatively low levels of resistance found to fluoroquinolones in this study is encouraging however continued monitoring of resistance prevalence in this population is an important aspect of antimicrobial resistance surveillance.

The significance of pork in human campylobacteriosis has not been defined. The majority of human cases (80-90%) are *C. jejuni* with only a small proportion due to *C. coli* [28-30]. Despite this, the high levels of resistance to macrolides and the moderate level of resistance to fluoroquinolones justifies continued research into antimicrobial resistance in *Campylobacter*. This research should address the prevalence of resistance, the source of resistance and the potential for genetic transfer of resistance to other *Campylobacter* species.

Salmonella

Methods

Isolation

AAFRD Laboratories Branch, Food Safety Division – Edmonton Alberta

Phase A samples were cultured by Agri-Food Laboratories Branch (AFLB), Food Safety Division of Alberta Agriculture, Food and Rural Development, Edmonton, AB. Samples were received pre-mixed within 24 hours of sample collection. Ten grams of feces were enriched in 90 ml of Buffered Peptone Water (BPW) at 35°C for 20-24 hours. From this, a sample was inoculated into both Rappaport Vassiliadis (RV) broth and Tetrathionate (TT) broth for selective enrichment. A sample of each of these broths was tested by PCR for the presence of *Salmonella*. A positive *Salmonella spp.* control and a negative *E. coli* control were set up at this point.

The RV broth and TT broth were each transferred to Xylose-Lysine-Tergitol 4 Agar (XLT4) and Rambach Agar (RAM) plates. The TT Broth was also transferred to Modified Semi-Solid Rappaport Vassiliadis – Difco (MSRV) media. All plates were incubated at 35°C for 18-24 hours, read and reincubated for 48 hours. The MSRV plate was subcultured onto the XLT4 and RAM for visual identification.

For samples which were PCR positive but culture negative, immunomagnetic separation technology (IMS) was used to increase sensitivity. Samples were enriched in TT broth with the Dynabeads® anti-*salmonella* beads (Dynal Biotech, ASA, Oslo, Norway) and cultured onto XLT4 and RAM plates. Positive samples were processed in the same manner as the positive cultures.

One suspect *Salmonella* colony per sample was selected for further characterization with the exception of cultures with colonies which appeared distinctly different. In those situations two colonies were selected. Samples were screened with Triple Sugar Iron Agar (TSI), Lysine Iron Agar (LIA), and ¼ MAC. Identification was confirmed with *Salmonella* Poly 0/01 agglutination. Atypical colonies were further tested with Vitek GNI or API-20E. *Salmonella* isolates were frozen at minus 70°C in defibrinated sheep's blood.

Frozen samples were sent to the Public Health Agency of Canada for serotyping as a final confirmation.

Prairie Diagnostic Services – University of Saskatchewan – Saskatoon, SK

Four herds were cultured by Prairie Diagnostic Services (PDS), Saskatoon, SK. The protocol developed by AFLB was utilized at PDS with a few modifications. PCR and IMS beads were not used. Positive confirmation of *Salmonella* differed slightly; one suspect colony per culture was incubated in TS broth and subsequently tested with TSI, urease, citrate, ¼ Blood Agar and ¼ MAC. Confirmation did not differ from AFLB. Positive isolates were sent in TS broth to the Saskatchewan Provincial laboratory for serotyping.

Laboratory Services Division – University of Guelph – Guelph, ON

Six herds were cultured by Laboratory Services Division (LSD), University of Guelph, Guelph, ON. Samples were received premixed at the laboratory within 24 hours of collection. A 25g sample was incubated with a pre-enrichment of BPW and Dynabeads® anti-*salmonella* beads (Dynal Biotech, ASA, Oslo, Norway). A positive control of *Salmonella thompson*, a negative control of *Escherichia coli* and a blank control were set up in parallel with the test. RV broth was used for selective enrichment. Following incubation, samples were plated onto selective agars Bismuth Sulfite and Brilliant Green Sulfa. Typical *Salmonella* colonies were streaked onto MacConkey's agar for purification. Suspect colonies were tested with TSI, LIA and urea to confirm *Salmonella* status. Isolates were frozen at -80°C in glycerol and sent to the Public Health Agency of Canada for serological testing.

Serotyping

Public Health Agency of Canada - Guelph, Ontario

Serological identification of *Salmonella* isolates cultured at AFLD and LSD was performed at the Public Health Agency of Canada, Office International des Épizooties (OIÉ) Reference Laboratory for Salmonellosis, in Guelph, Ontario, Canada. The O or somatic antigens of the *Salmonella* isolates were determined by slide agglutination [31]. The H or flagellar antigens were identified using a microtechnique [32] that employs

microtitre plates. The antigenic formulae of Le Minor and Popoff [33] were used to name the serovars.

The standard phagetyping technique described by Anderson and Williams (1956) [34] was followed. *Salmonella* Enteritidis strains were phagetyped with typing phages obtained from the International Centre for Enteric Phage Typing (ICEPT), Central Public Health Laboratory, Colindale, United Kingdom [35] via the National Microbiolgoy Laboratory (NML) by the Public Health Agency of Canada, Winnipeg, Manitoba. The phagetyping scheme and phages for *Salmonella* Typhimurium, developed by Callow [36] and further extended by Anderson [37] and Anderson and colleagues [38] were obtained from the ICEPT via the NML. The *Salmonella* Heidelburg phagetyping scheme and phages were supplied by the NML [39]. Isolates that reacted with the phages but did not conform to any recognized phage type were considered atypical (AT). Strains which did not react with any of the typing phages were considered untypable (UT).

Saskatchewan Provincial Laboratory – Regina, Saskatchewan

Serological identification of *Salmonella* isolates cultured at PDS was performed at the Saskatchewan Provincial Laboratory, Regina, Saskatchewan. The O or somatic antigens of the *Salmonella* isolates were determined by slide agglutination [40]. The H or flagellar antigens were identified by broth culture method [40]. The antigenic formulae of Le Minor and Popoff [33] were used to name the serovars. Isolates needing further identification were sent to the National Laboratory of Enteric Pathogens in Winnipeg, Manitoba. Samples identified as *S.* Typhimurium (inc. var. Copenhagen) and *S.* Heidelburg were sent to the Public Health Agency of Canada, Office International des Épizooties (OIÉ) Reference Laboratory for Salmonellosis, in Guelph, Ontario, Canada and typed following the methods described above.

Antimicrobial Sensitivity testing – Broth Microdilution

AAFRD Laboratories Branch, Food Safety Division – Edmonton Alberta

Prairie Diagnostic Services – University of Saskatchewan – Saskatoon, SK

All isolates were tested for antimicrobial susceptibility using a broth microdilution technique following Clinical and Laboratory Standards Institute (CLSI) guidelines [41, 42]. Sensititre custom designed CMV7CNCD minimum inhibitory concentration (MIC) panels (Sensititre® TREK Diagnostic Systems, Westlake, Ohio) were used. Each isolate was grown up on a non-selective media. A 0.5 McFarland standard was made in 5 ml of demineralized water of which 10 µl was transferred into 10 ml of Mueller Hinton broth with TES. 50 µl was inoculated into each of the 96 wells on the panel. Inoculated plates were incubated and read by the ARIS/AutoReader automated system. The breakpoint concentrations of the antimicrobials were based on CLSI approved standards [41, 42]. Readings were transferred to the SAMS computer software where they were interpreted according to CLSI guidelines. Quality control organisms used were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeroginosa* ATCC 27853. AFLB preformed the antimicrobial sensitivity testing on the isolates from phase A herds while PDS tested isolates from phase B herds.

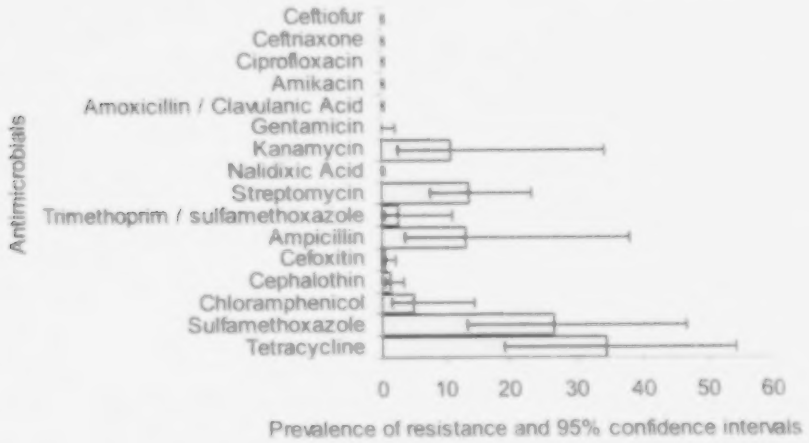
Antimicrobial Sensitivity Results

Individual Drug Resistance:

Of the 468 *Salmonella* isolates, 293 (63%) were susceptible to all antimicrobials tested. No resistance was seen to amikacin, amoxicillin-clavulanic acid, ceftiofur, ceftriaxone, ciprofloxacin, or nalidixic acid (Figure 10). After adjusting for herd level clustering the highest levels of resistance were seen to tetracycline (35%), sulfamethoxazole (27%), ampicillin (13%), streptomycin (14%) and kanamycin (11%). Lower levels of resistance were seen to chloramphenicol (5%), trimethoprim-sulfamethoxazole (3%), cefoxitin (1%)

and cephalothin (1%). One isolate was resistant to gentamicin. The distributions of the Minimum Inhibitory Concentration (MIC) values for each antimicrobial tested are presented as the percentage of isolates (Figure 12).

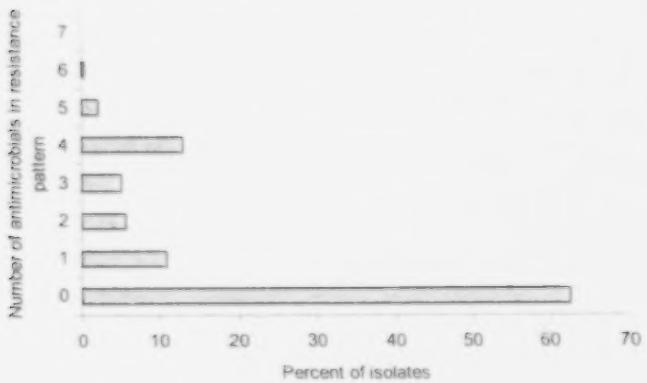
Figure 10. Individual antimicrobial drug resistance in porcine fecal *Salmonella* isolates



Multiple Drug Resistance:

Of the 468 isolates, 37% were resistant to at least one antimicrobial and 26.5% were resistant to two or more. Resistance to four antimicrobials was the most common multiple drug resistance and was seen in 13% of the isolates (Figure 11).

Figure 11. Multiple Drug Resistance levels in porcine *Salmonella* isolates



***Salmonella* Antimicrobial resistance patterns**:

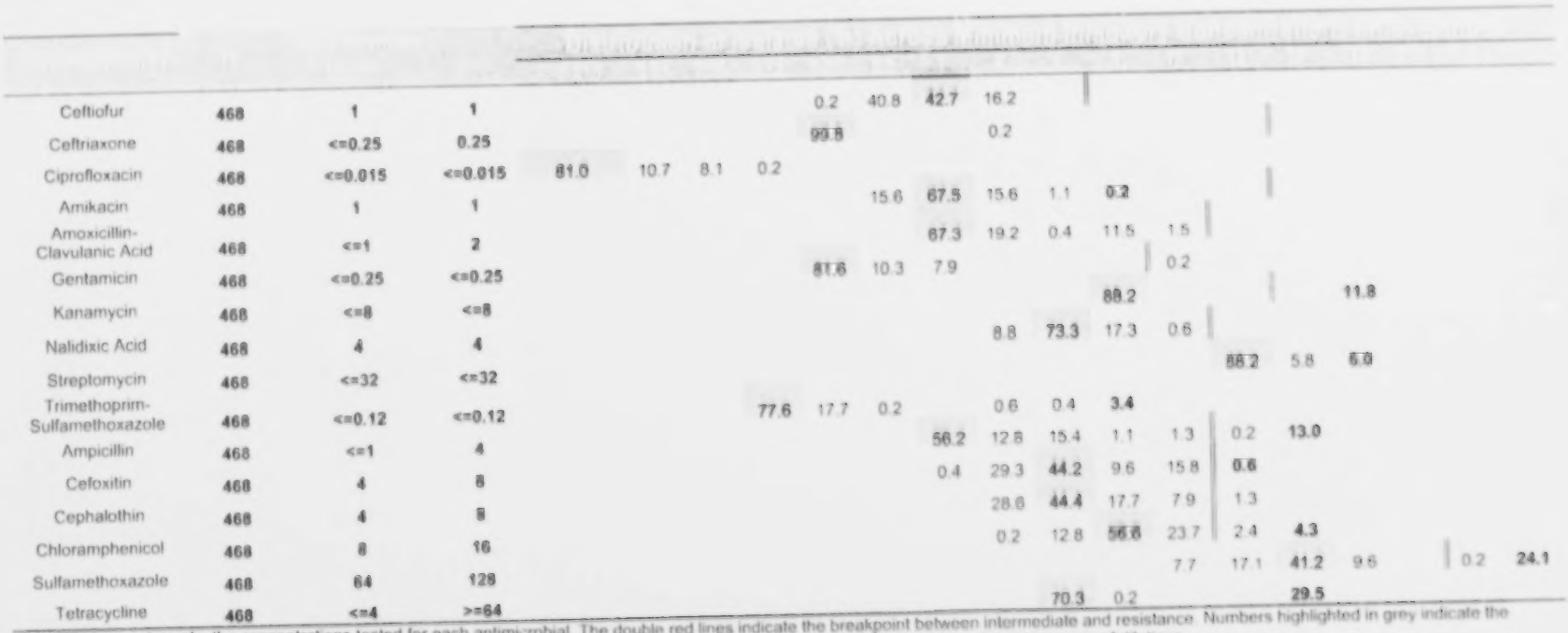
Twenty nine different antimicrobial resistance patterns were found. The most common resistance pattern was to ampicillin, kanamycin, sulfamethoxazole and tetracycline and was seen in forty-five isolates (Table 9).

Table 9. Antimicrobial resistance patterns

AMR Pattern [a]	Total	%	AMR Pattern [a]	Total	%
AMP-KAN-SMX-TCY	45	9.6	AMP-SMX	2	0.4
TCY	36	7.7	AMP	2	0.4
STR-SMX-TCY	14	3	FOX-CEP-CHL-STR-SMX-SXT	1	0.2
STR-TCY	14	3	AKSSuT+GEN	1	0.2
CHL-STR-SMX-TCY	7	1.5	CEP-CHL-STR-SMX-SXT	1	0.2
CHL	7	1.5	STR-SMX-TCY-SXT	1	0.2
AKSSuT	6	1.3	AMP-KAN-STR-SMX	1	0.2
CHL-STR-SMX-SXT	6	1.3	CEP-CHL-SMX-SXT	1	0.2
SMX-TCY	6	1.3	CHL-STR-SMX-SXT	1	0.2
SMX-TCY-SXT	4	0.9	CHL-STR-SMX	1	0.2
CHL-SMX	4	0.9	AMP-STR-SMX	1	0.2
SMX	4	0.9	SMX-SXT	1	0.2
CEP-AMP-KAN-SMX-TCY	2	0.4	CEP	1	0.2
AMP-SMX-TCY	2	0.4	FOX	1	0.2
CHL-SMX-SXT	2	0.4			

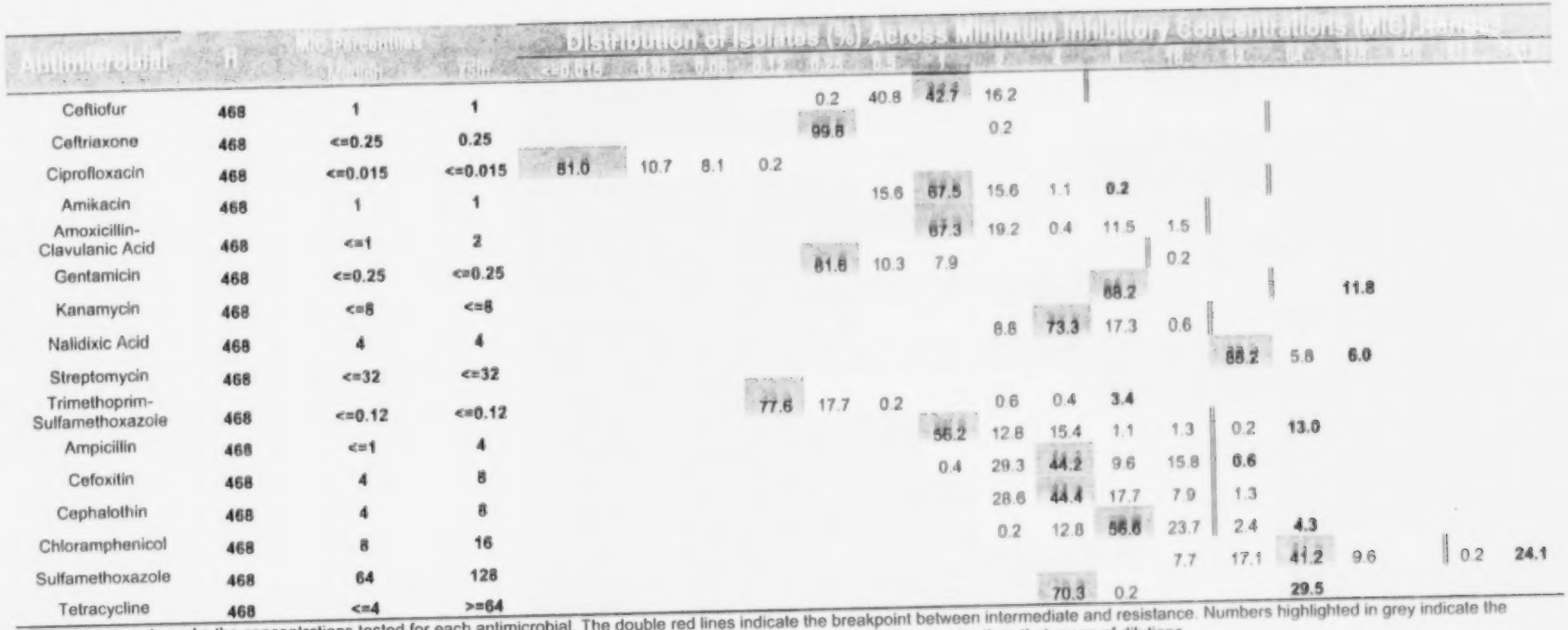
[a] AKSSuT: ampicillin, kanamycin, streptomycin, sulfamethoxazole tetracycline; AMP: ampicillin; CEP: cephalothin; CHL: chloramphenicol FOX: cefoxitin; KAN: kanamycin; SMX: sulfamethoxazole; STR: streptomycin; SXT: trimethoprim-sulfamethoxazole; TCY: tetracycline

Figure 12. MIC distributions for porcine *Salmonella* isolates



The white area demarks the concentrations tested for each antimicrobial. The double red lines indicate the breakpoint between intermediate and resistance. Numbers highlighted in grey indicate the median. Bold font numbers indicate isolates with growth in all wells within the tested range, indicating the actual MIC is greater than that range of dilutions.

Figure 12. MIC distributions for porcine *Salmonella* isolates


Antimicrobial
N
MIC Percentiles
Distribution of Isolates (%) Across Minimum Inhibitory Concentrations (MIC) Ranges
Ceftiofur 468 1 1
Ceftriaxone 468 <=0.25 0.25
Ciprofloxacin 468 <=0.015 <=0.015
Amikacin 468 1 1
Amoxicillin-Clavulanic Acid 468 <=1 2
Gentamicin 468 <=0.25 <=0.25
Kanamycin 468 <=8 <=8
Nalidixic Acid 468 4 4
Streptomycin 468 <=32 <=32
Trimethoprim-Sulfamethoxazole 468 <=0.12 <=0.12
Ampicillin 468 <=1 4
Cefoxitin 468 4 8
Cephalothin 468 4 8
Chloramphenicol 468 8 16
Sulfamethoxazole 468 64 128
Tetracycline 468 <=4 >=64
0.2 40.8 42.7 16.2
99.8 0.2
81.0 10.7 8.1 0.2
15.6 67.5 15.6 1.1 0.2
67.3 19.2 0.4 11.5 1.5
81.6 10.3 7.9 0.2
88.2 11.8
8.8 73.3 17.3 0.6
88.2 5.8 6.0
77.6 17.7 0.2 0.6 0.4 3.4
55.2 12.8 15.4 1.1 1.3 0.2 13.0
0.4 29.3 44.2 9.6 15.8 0.6
28.6 44.4 17.7 7.9 1.3
0.2 12.8 56.6 23.7 2.4 4.3
7.7 17.1 41.2 9.6 0.2 24.1
70.3 0.2 29.5

The white area demarks the concentrations tested for each antimicrobial. The double red lines indicate the breakpoint between intermediate and resistance. Numbers highlighted in grey indicate the median. Bold font numbers indicate isolates with growth in all wells within the tested range, indicating the actual MIC is greater than that range of dilutions.

Thirty serovars were identified with a range of 2 to 12 serovars per herd. All serovars with more than ten isolates were found in at least two herds (Table 10). Serovars with more than 10 isolates were found in all phases of production with the exception of *Salmonella banana* which was isolated from herds with only grow-finish animals sampled (Table 11). Phage typing results were not available at the time of this report.

Table 10. Frequency of ten most common serovars and number of source herds

Serovar	Number of Isolates	Number of herds
Derby	124	6
Typhimurium var. Copenhagen	81	6
Putten	49	3
Infantis	37	6
I:ROUGH-O:d:I,w	34	3
Banana	27	2
Mbandaka	26	5
Anatum	20	3
Give	19	4
Other (20 serovars)	51	

Table 11. Distribution of phase of production from which most common serovars were isolated

Serovar	Nursery	Grow-finish	Sows
Derby	6	83	35
Typhimurium var. Copenhagen	24	28	29
Putten	7	25	17
Infantis	4	22	11
I:ROUGH-O:d:I,w	15	11	8
Banana*	0	27	0
Mbandaka	14	12	0
Anatum	3	9	8
Give	1	3	15
Other (20 serovars)	9	21	21

* only isolated from Phase B herds therefore only grow-finish pigs sampled

Conclusions

This study found a slightly higher proportion (62.6%) of isolates susceptible to all antimicrobial in comparison to other Canadian reports (38% to 53.4%) [6, 7, 43-45] and notably higher than reports from the United States [46-48]. Resistance to two or more antimicrobial was found in 26.5% of isolates which was lower than the 42% reported in Ontario [6] but similar to the 28.6% and reported from on-farm samples from finisher pigs in Alberta [44].

Isolates were most commonly resistant to tetracycline and sulfamethoxazole which was similar to other Canadian reports however the prevalence of streptomycin resistance (13.6%) was lower than the previously reported (25 – 50%) [6, 43, 44]. The finding of no, or very low, resistance to ceftiofur, ceftriaxone, ciprofloxacin, amikacin, amoxicillin-clavulanic acid, nalidixic acid, gentamicin and cefoxitin are also in agreement with other Canadian reports [6, 7, 43-45].

Salmonella isolates were collected at similar rates from all phases of production (25 – 38%) with the exception of pooled sow samples (51%). The ten most common serovars were found in all phases of production with the exception of *S. bananа* which was isolated from Phase B herds in which only grow-finish pigs were sampled.

Escherichia coli

Methods

Isolation

Prairie Diagnostic Services – University of Saskatchewan – Saskatoon, SK

Samples were sent premixed to the lab and cultured for *Escherichia coli* within 24 hours of collection. A heavily coated cotton swab was streaked onto whole Blood Agar and MacConkey plates and incubated at 37°C for 18 hours. Three lactose-positive colonies were selected from each MacConkey plate and incubated in trypticase soy (TS) broth for 3-4 hours at 37°C. Samples with both hemolytic and non-hemolytic colonies had three colonies from each type selected. Each TS broth was inoculated onto urea and citrate slants with a 1 µl loop and a blood agar plate was concurrently inoculated to ensure sample purity. These were then incubated at 37°C for 18 hours. Samples requiring further confirmation were tested with Triple Sugar Iron / Indole. Pure, confirmed *E. coli* cultures were frozen in 25% glycerol at -80°C.

Antimicrobial Sensitivity testing – Broth Microdilution

AAFRD Laboratories Branch, Food Safety Division – Edmonton Alberta

Prairie Diagnostic Services – University of Saskatchewan – Saskatoon, SK

The methods used to test the *E. coli* isolates was the same as the *Salmonella* isolates. AAFRD completed antimicrobial sensitivity testing on 729 isolates obtained from the Phase A herds which were cultured at PDS and delivered frozen to AAFRD. PDS completed antimicrobial testing on 710 isolates from the phase B herds.

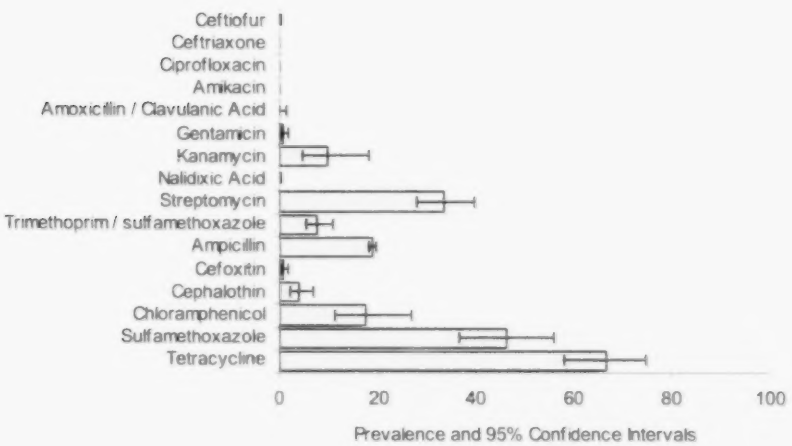
Antimicrobial Sensitivity Results

A total of 405 samples were cultured for *E. coli* resulting in 1439 isolates. An average of 63 isolates was saved per herd (range; 60 to 88).

Individual Antimicrobial Drug Resistance:

No resistance was identified to amikacin, ceftriaxone, or ciprofloxacin in the 1439 isolates (Figure 13). The highest levels of resistance were seen to tetracycline (67%), sulfamethoxazole (46%), streptomycin (33%), ampicillin (18%) and chloramphenicol (17%). Moderate levels of resistance were seen to kanamycin (9%), trimethoprim-sulfamethoxazole (7%) and cephalothin (4%). One percent or less of the isolates were resistant to amoxicillin-clavulanic acid, cefoxitin, ceftiofur, gentamicin and nalidixic acid.

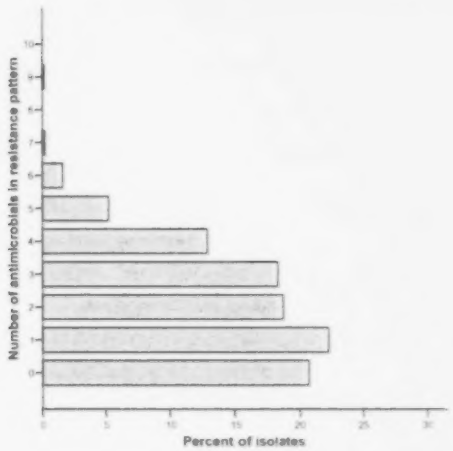
Figure 13. Individual antimicrobial drug resistance in porcine fecal *E. coli* isolates adjusted for clustering within herd



Multiple Drug Resistance:

Of the 1439 isolates tested, 298 (20.7%) were susceptible to all antimicrobials (Figure 15). Twenty-two percent were resistant to one antimicrobial and 57.1% were resistant to 2 or more antimicrobials. Resistance to two antimicrobials was the most common multiple drug resistance pattern and was seen in 18.7% of the isolates. Two isolates were resistant to nine antimicrobials.

Figure 15. Multiple drug resistance in porcine *E. coli* isolates



Antimicrobial Resistance Patterns:

Ninety-two different AMR patterns were identified in the 1439 *E. coli* isolates, of which the twenty most common are presented (Table 12). Resistance to tetracycline alone was the most common pattern and was seen in 16.3% of the isolates. The most common multiresistant pattern was to sulfamethoxazole and tetracycline which was seen in 6.8% of the isolates. The penta-resistance pattern, with or without other antimicrobials, ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfamethazine and tetracycline) was seen in 1.1% of isolates and AKSSuT (ampicillin, kanamycin, streptomycin, sulfamethazine and tetracycline) in 1.6%. ACKSSuT was seen in 0.6% of isolates (TTable 13).

Table 12. Twenty most common antimicrobial resistance patterns in *E. coli* isolates

AMR Pattern [a]	Total	%
None	298	20.7
TCY	235	16.3
SMX-TCY	98	6.8
STR-SMX-TCY	97	6.7
STR-TCY	94	6.5
CHL-STR-SMX-TCY	56	3.9
CHL-SMX-TCY	45	3.1
STR	36	2.5
SMX	35	2.4
AMP-STR-TCY	34	2.4
AMP-TCY	31	2.2
AMP-STR-SMX-TCY	23	1.6
CHL-SMX	20	1.4
KAN-STR-SMX-TCY	19	1.3
CHL-KAN-SMX-TCY	17	1.9
AMP-SMX-TCY-SXT	16	1.1
AMP-SMX-TCY	15	1.0
CHL-KAN-SMX	15	1.0
SMX-TCY-SXT	15	1.0
AMP-STR-SMX-TCY-SXT	14	1.0

[a] AMP: ampicillin; CHL: chloramphenicol; KAN: kanamycin; SMX: sulfamethoxazole; STR: streptomycin; SXT: trimethoprim-sulfamethoxazole; TCY: tetracycline

Table 13. Frequency of Penta-resistance patterns ACSSuT and AKSSuT with or without additional antimicrobials

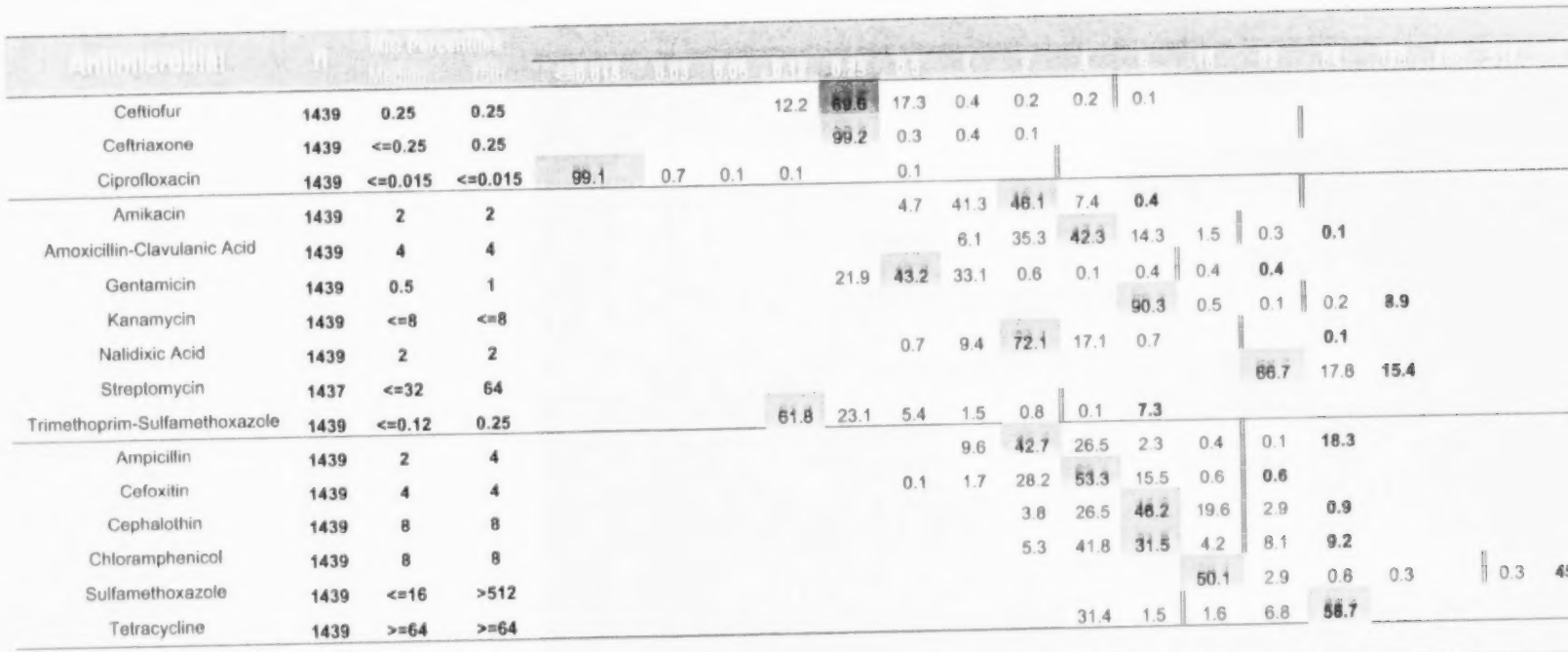
AMR Pattern [a]	Total	%
AKSSuT	12	0.8
ACKSSuT	6	0.4
ACSSuT	6	0.4
ACSSuT+SXT	6	0.4
ACKSSuT+GEN	2	0.1
ACSSuT+CEP	2	0.1
AKSSuT+AMC+FOX+CEP+SXT	1	0.1
AKSSuT+FOX+CEP	1	0.1
AKSSuT+FOX+CEP+GEN+NAL	1	0.1

[a] AKSSuT: ampicillin, kanamycin, streptomycin, sulfamethoxazole tetracycline ACSSuT: ampicillin, chloramphenicol, streptomycin, sulfamethoxazole tetracycline AMC: amoxicillin-clavulanic acid; CEP: cephalothin; FOX: cefoxitin; GEN: gentamicin; NAL: nalidixic acid

Minimum Inhibitory Concentration Distributions:

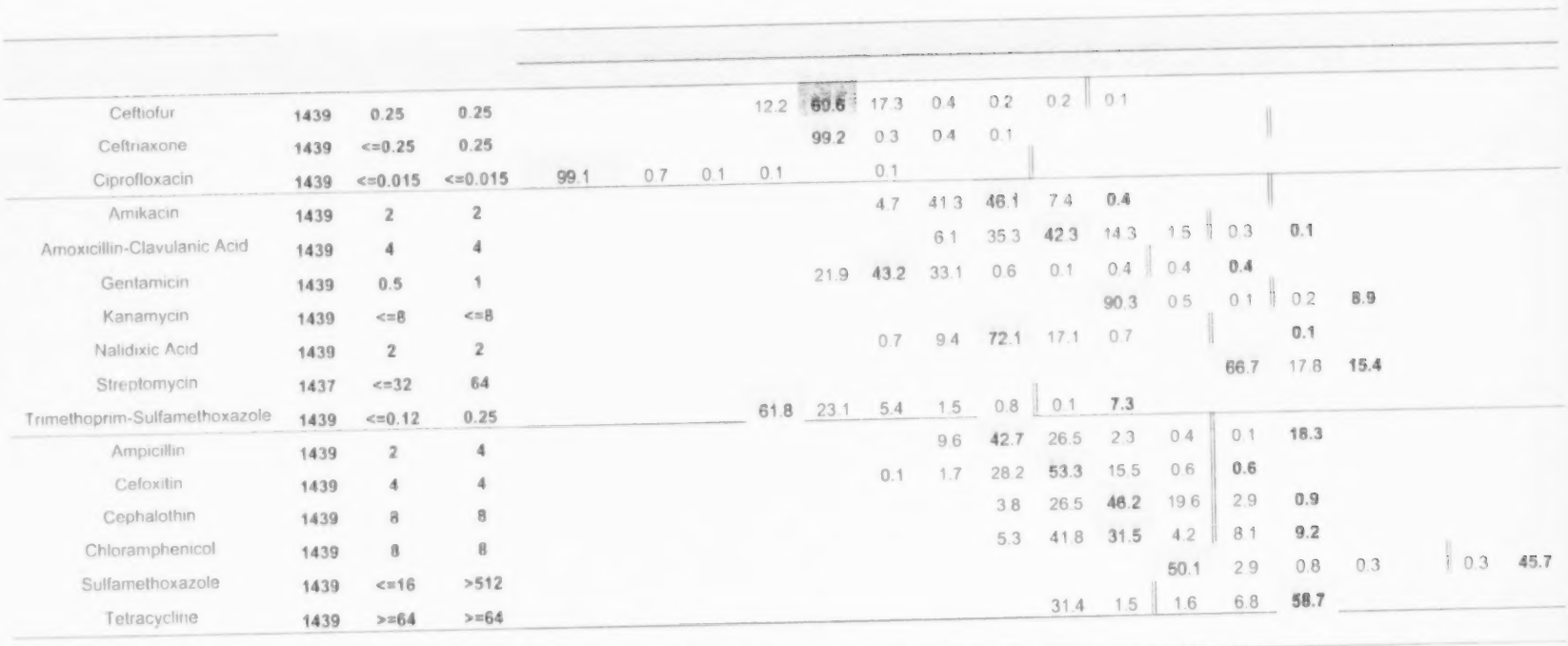
The distributions of the Minimum Inhibitory Concentration (MIC) values for each antimicrobial tested are presented (Figure 16) as the percentage of isolates.

Figure 16. Minimum Inhibitory Concentration (%) distributions for porcine *E. coli* isolates


Ceftiofur 1439 0.25 0.25
Ceftriaxone 1439 <=0.25 0.25
Ciprofloxacin 1439 <=0.015 <=0.015
Amikacin 1439 2 2
Amoxicillin-Clavulanic Acid 1439 4 4
Gentamicin 1439 0.5 1
Kanamycin 1439 <=8 <=8
Nalidixic Acid 1439 2 2
Streptomycin 1437 <=32 64
Trimethoprim-Sulfamethoxazole 1439 <=0.12 0.25
Ampicillin 1439 2 4
Cefoxitin 1439 4 4
Cephalothin 1439 8 8
Chloramphenicol 1439 8 8
Sulfamethoxazole 1439 <=16 >512
Tetracycline 1439 >=64 >=64

Note: The white fields indicate the range tested for each antimicrobial. Vertical double bars mark the breakpoint between intermediate and resistant. The darkened cells locate the median. Bold font numbers indicate isolates with growth in all wells within the tested range, indicating the actual MIC is greater than that range of dilutions

Figure 16. Minimum Inhibitory Concentration (%) distributions for porcine *E. coli* isolates



Note: The white fields indicate the range tested for each antimicrobial. Vertical double bars mark the breakpoint between intermediate and resistant. The darkened cells locate the median. Bold font numbers indicate isolates with growth in all wells within the tested range, indicating the actual MIC is greater than that range of dilutions

Conclusions

Antimicrobial resistance profiles and patterns were very similar to those reported from surveillance of hogs in Canadian abattoirs in 2002 and 2003 [7, 45]. This suggests that on-farm and abattoir sampling may be complementary methods for monitoring AMR in swine. The collection of abattoir samples allows for a larger sampling frame and decreased biosecurity risk to involved herds while on-farm data allows for the collection of pen and herd level information such as antimicrobial use, animal movement and hygiene practices. Examining variation in these risk factors at the herd or pen level has the potential to improve understanding of the source and persistence of antimicrobial resistance.

The prevalence of resistant isolates was highest to tetracycline, sulfamethoxazole and streptomycin as has been reported by other national surveillance programs [8, 45, 49]. As well, sensitivity results for the antimicrobials of greatest interest in human medicine reflected expectations based on previous studies. No resistance was seen to ceftriaxone, less than one percent of isolates were resistant to ceftiofur and 3.8% were resistant to cephalothin. No resistance was seen to the fluoroquinolones, ciprofloxacin and nalidixic acid. These results are in agreement with surveillance results from Denmark, the United States and Canada. In contrast, Spain has shown higher resistance to many antimicrobials demonstrating the value of continued antimicrobial resistance monitoring [50].

Associations between antimicrobial use and resistance

Purpose

This aspect of the project investigated the hypothesis that antimicrobial resistance is associated with antimicrobial use. The primary hypothesis was antimicrobial resistance would increase with antimicrobial treatment incidence regardless of the product administered. The secondary hypothesis was antimicrobial resistance is associated with increased antimicrobial treatment incidence of products within the same family of antimicrobials. These hypotheses were investigated simultaneously by modeling the association between pen level *E. coli* antimicrobial resistance and herd antimicrobial treatment incidence for all products and for products in the same family as the outcome antimicrobial of interest.

Methods

Antimicrobial resistance was defined as resistance in any *E. coli* isolate from a pen to the antimicrobial of interest. Separate models were developed with resistance to tetracycline and sulfamethoxazole as the outcome of interest. Binomial models accounted for herd level clustering using generalized estimating equations (PROC GENMOD, SAS for Windows ver. 8.2, SAS Institute Inc., Cary, NC, USA) with an exchangeable correlation structure.

Antimicrobial treatment incidence was quantified for each herd. A 'total treatment incidence' for all phases of production, routes of administration and products administered was calculated for each herd. Each herd's total use was also stratified by phase of production (regardless of route of administration) and by route of administration (regardless of phase of production). Treatment incidence was also stratified by phase and route.

Family specific antimicrobial treatment incidence was defined as the treatment incidence through feed or water for antimicrobials in the tetracycline or sulfonamide family.

Tetracycline treatment incidence included oxytetracycline and chlortetracycline administration via feed and tetracycline hydrochloride administration via water. Sulfonamide treatment incidence included the administration of sulfamethazine via feed and any sulfonamide via water. Data limitations precluded examining family specific use of injectable antimicrobials.

Univariate models examined the association between antimicrobial resistance and treatment incidence with all products. Models examined total treatment incidence, treatment incidence by each route (for all phases), treatment incidence for each phase (via all routes) and for treatment incidence for each route/phase combination. No study herds used water medication in suckling piglets and therefore this combination was not examined. The univariate associations between resistance and family specific treatment incidences were examined in a similar fashion.

Univariate models with a Z-test p-value ≤ 0.05 were considered for inclusion in the multivariate analysis. As the various treatment incidences were calculated by stratification of data, significant variables were examined for correlation to avoid collinearity problems in multivariate models. Variables with a Pearson correlation coefficient ≥ 0.75 had the more general of the two variables dropped from consideration. Treatment incidence which specified route was considered more specific than treatment incidence for all routes. Treatment incidence which specified the family of antimicrobial was considered more specific that treatment incidence for all products. To determine if treatment incidence for a specific class or all classes combined should be selected, the variable with a greater difference in AMR prevalence between no use and use at the upper quartile was included.

All remaining significant variables were combined in a multivariate model. Non-significant variables (p-value > 0.05) were removed from the model one at a time, starting with the largest p-value until all variables were significant.

From this model, biologically plausible interactions were considered and maintained in the model if $p \leq 0.05$. Lastly, the role of management variables as confounders on the relationship between antimicrobial use and resistance were examined by addition to the model one at a time. Confounding was considered to occur if the odds ratio of any variable in the model changed by more than 20%.

The herd level management variables considered were; **farm type** (commercial or breeding herd), **all in all out** management of the grow-finish area, number of **sites** the barn was located on (1 to 3), number of times the entire grow-finish area of the barn was **washed** in previous year, if grow-finish pens were routinely **emptied** of pigs before new pigs were added, if grow-finish pens were routinely cleaned to a **manure free** state between batches of pigs, if grow-finish pens were washed and **disinfected** between batches of pigs and the typical amount of time grow-finish pens were left empty between batches of pigs (**downtime** in days). The pen level variables considered as confounders were; the **sex** of the pigs (boars, gilts, barrows or mixed), the **duration** pigs had been in the pen at sampling (weeks), the number of animals per square meter of pen (**stocking density**), a **pen hygiene score**, the **floor type** (full slat, partial slat or solid floor) and if feeders were used or if pigs were **floor fed**.

If more than one herd management variable was found to be a confounder a model was constructed with all potential confounders and variables of primary interest (AMU). Variables which were no longer significant ($p \geq 0.05$) were removed from the model one at a time while monitoring the relative change in the remaining variables.

Results

General

Resistance

An average of 20.3 pens was sampled (range, 19 to 25) per herd in 20 herds resulting pen 405 pen samples. On average each pen contributed 3.6 isolates (range, 1 to 6) resulting in 1439 isolates.

Herd and Management Descriptive Statistics

Seven of the herds sold breeding stock while the remaining thirteen were commercial pig producers. Nine practiced all-in-all-out management by room in the grow finish area. Fifteen herds were located on one site, two on two sites and three on three sites. On average, the entire grow-finish area of the barn was washed 2.9 times a year (range, 0 to 17). Twelve herds routinely emptied pens of pigs before bringing in new animals, ten herds routinely cleaned pens to a manure free state and eight herds routinely washed and disinfected pens before placing a new batch of pigs in the pen. On average pens were left empty for 1.4 days between batches of pigs (s=1.7).

Pen Level Descriptive Statistics

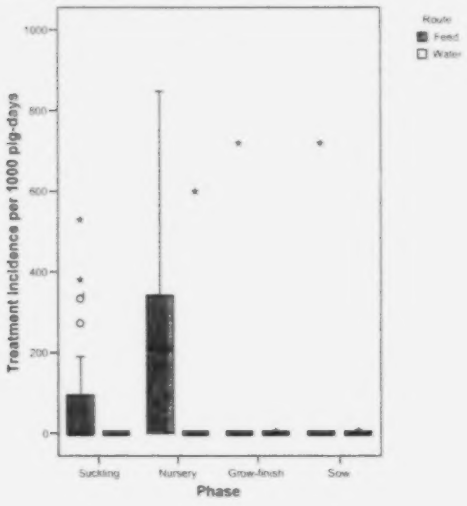
Five percent of the pens sampled contained boars, 41% gilts, 33% barrows and 20% mixed sex. On average, pigs had been in the pen for 5.9 weeks at the time of sampling (s = 4.1) and the average number of pigs per square meter was 1.7 (s = 0.78). A the time of sample collection 21% of the pens were considered very clean, 56% had ¼ of the pen which was dirty, 14% had ½ of the pen which was dirty and 10% had more than ½ of the pen which was dirty. Twenty-five percent of the pens sampled had a fully-slatted floor, 74% a partially slatted floor and 1% a solid floor. Eighty-nine percent of the pens sampled had feeders while 11% were floor-fed.

Tetracycline resistance

Tetracycline antimicrobial use

Tetracycline was administered to groups of pigs in the previous 12 months in fifteen of the twenty study herds. All herds which reported group tetracycline medication administered it through feeds while three herds administered it through both feed and water. Of the three herds which administered tetracycline via water, one herd administered it to grow-finish pigs at a treatment incidence of 5.4 treatments per 1000 pig-days, one to sows at 6.8 treatments per 1000 pig-days and one to nursery pigs at 600.0 treatments per 1000 pig-days. Tetracyclines were most commonly used in nursery pigs (mean, 276) followed by suckling pigs, grow-finish pigs and sows (Figure 17).

Figure 17. Distribution of treatment incidence per 1000 pig-days for antimicrobials in the tetracycline family



Tetracycline Resistance

Of the 1439 isolates examined, 66.8% were resistant to tetracycline (95% CI, 57.8 to 74.7). Twenty one isolates were of intermediate sensitivity. When examined at the pen level, 345 of the 405 pens sampled had at least one resistant isolate (85.2%) (s= 12.9). Five herds had all pens considered resistant to tetracycline.

Final Model

A final multivariable model of tetracycline resistance found nursery feed tetracycline treatment incidence and sow feed treatment incidence for all products to be significant. First order interactions were not significant and no herd management variables were important confounders. Administering tetracycline via feed to nursery pigs for 10,000 pig-days resulted in a 2.3% increase in the odds of at least one *E. coli* isolate from a pen of grow-finish pigs being resistant to tetracycline. For every 10,000 pig-days of feed medication administered to sows the odds of resistance to tetracycline by increased 33% (95% CI, 1.9 to 73.6%) in at least one *E. coli* isolate from a pen of grow-finish pigs (Table 14).

Table 14. Final model of the association between tetracycline resistance and antimicrobial treatment incidence per 10,000 pig-days (n=405 pens)

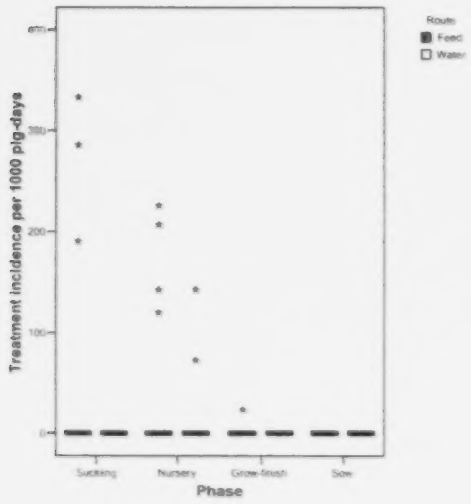
				95% Confidence interval		
Route	Phase	Family	OR	Lower	Upper	*P*-value
Feed	Nursery	Tetracycline	1.02	1.00	1.05	0.03
Feed	Sow	All	1.33	1.02	1.74	0.04

Sulfamethoxazole resistance

Sulfonamide antimicrobial use

Seven herds administered sulfonamide antimicrobials to groups of pigs within the 12 months prior to sample collection. Five herds administered sulfonamides via feed and two through water. Water administration of sulfonamides occurred only in nursery pigs. Sulfonamides were administered to nursery pigs in 4 herds (mean, 45.5; s=77.0) to suckling piglets in 3 herds (mean 40.4; s=101.6) and to grow-finish pigs in one herd (23.6 treatments per 1000 pig-days) (Figure 18). No herds administered sulfonamides to groups of sows.

Figure 18. Distribution of sulfonamide treatment incidence via feed and water across study herds



Sulfamethoxazole Resistance

Of the 1439 isolates examined, 46.0% were resistant to sulfamethoxazole (95% CI, 36.4 to 56.0). No isolates were of intermediate sensitivity. When examined at the pen level, 273 of the 405 pens had at least one isolate resistant to sulfamethoxazole (67%). Herds ranged from having 26.3 to 100% of pens considered resistant.

Final model

A final model found that feeding antimicrobials to suckling pigs for 10,000 pig-days increased the odds by 2.5% of pen level sulfamethoxazole resistance occurring in *E. coli*. Controlling for other variables in the model, feeding grow-finish pigs antimicrobials for 10,000 pig-days increased the odds by 1.4% pen *E. coli* sulfamethoxazole resistance occurring. Administering antimicrobials to sows via water for 10,000 pig-days increased the odds by 16.1 times of grow-finish pen *E. coli* resistance to sulfamethoxazole occurring (Table 15).

Table 15. Final model of the association between sulfamethoxazole resistance and antimicrobial treatment incidence per 10,000 pig-days (n=405 pens).

				95% Confidence interval		
Route	Phase	Family	OR	Lower	Upper	*P*-value
Feed	Suckling	All	1.03	1.01	1.04	0.004
Feed	Grow-finish	All	1.01	1.01	1.02	0.001
Water	Sow	All	16.1	5.70	45.5	<.0001

Conclusions

E. coli was selected as the organism of interest as it is commonly used as an indicator organism, is highly prevalent, easily isolated and lends itself to comparisons between species, across time and space. The treatment incidence for all products, in addition to the family specific product, was investigated due to potential cross selection of resistance genes due to genetic linkage. The frequency of antimicrobial in the herd was associated with the occurrence of antimicrobial resistance.

The models developed are unique in that no other studies have quantified herd level antimicrobial use in all stages of production for this duration of time. We hypothesized that long term use is important in the development and persistence of resistance and that antimicrobial use in the entire production chain may impact the levels of resistance seen in the grow-finish barn. This has been supported by other's work which found, for certain antimicrobials, feed antimicrobial use in nursery and suckling piglets was positively associated with resistance in the grow-finish barn [51].

Interestingly, family specific use was not necessarily more strongly associated with resistance than a general treatment incidence for all products as was demonstrated by the model for sulfamethoxazole resistance. It has been previously reported that tetracycline use in grow-finish rations was the only significant predictor for sulfisoxazole resistance [51]. As well, no phase of production or route of administration was consistently associated with an increase in resistance suggesting that the development and persistence of resistance is complex.

Further work is needed in this area of study and model validation is ongoing. These results are preliminary and should be interpreted cautiously at this stage.

Conclusions and Recommendations

This project has described the antimicrobial resistance profiles of *Salmonella*, *Campylobacter* and *Escherichia coli* from grow-finish pigs in Saskatchewan and Alberta. This information had not previously been described and similarities and differences between other countries and regions were discussed. The project also quantified the antimicrobial use on study farms in the 12 months prior to sample collection in all phases of production. Prior to this, only quantitative descriptions of antimicrobial use were available for Western Canada. Lastly, the association between antimicrobial resistance and use at the farm level was examined. Further work is pending in this area however preliminary results suggest a positive association between antimicrobial use and resistance.

The information generated by this project will be useful to the Canadian Integrated Program for Antimicrobial Resistance (CIPARS) APF working group in their future efforts to collect antimicrobial use and resistance information from Canadian swine farms. Additionally, the data found in this project will be useful to both industry and government personnel whom are addressing the issues of emerging antimicrobial resistance and prudent antimicrobial use in the swine industry.

Further research is being conducted to examine the association between phenotypic and genotypic resistance and genotype and virulence in a subset of *E. coli* isolates from this project.

Acknowledgements

The department's support has been acknowledged with all presentation of this data. To date, this has included presentation in poster format within the Western College of Veterinary Medicine and oral format at the annual conference of the Canadian Association of Veterinary Epidemiology and Preventive Medicine. Plans exist to present the data at a provincial, regional and national conference this year. In addition, the department will be acknowledged for its support in any papers which are accepted for publication based on this data. Producers and veterinarians participating in the project were made aware of the funding provided by Saskatchewan Department of Agriculture, Food and Rural Revitalization.

Literature Cited

1. Lee, L.A., N.D. Puhr, E.K. Maloney, N.H. Bean, and R. Tauxe, *Increase in Antimicrobial-Resistant Salmonella Infections in the United States, 1989-1990.* J Infect Dis, 1994. **170**: p. 128-134.
2. Barza, M., *Potential Mechanisms of Increased Disease in Humans from Antimicrobial Resistance in Food Animals.* Clin Infect Dis, 2002. **34**(S3): p. S123-5.
3. Travers, K. and M. Barza, *Morbidity of Infections Caused by Antimicrobial-Resistant Bacteria.* Clin Infect Dis, 2002. **34**(S3): p. S131-4.
4. Schwarz, S., C. Kehrenberg, and T.R. Walsh, *Use of antimicrobial agents in veterinary medicine and food animal production.* Int J Antimicrob Agents, 2001. **17**(6): p. 431-437.
5. Dunlop, R.H., S.A. McEwen, A.H. Meek, W.D. Black, R.M. Friendship, and R.C. Clarke, *Prevalences of resistance to seven antimicrobials among fecal Escherichia coli of swine on thirty-four farrow-to-finish farms in Ontario, Canada.* Prev Vet Med, 1998. **34**(4): p. 265-82.
6. Larkin, C., C. Poppe, B. McNab, B. McEwen, A. Mahdi, and J. Odumeru, *Antibiotic resistance of Salmonella isolated from hog, beef, and chicken carcass samples from provincially inspected abattoirs in Ontario.* J Food Protect, 2004. **67**(3): p. 448-55.
7. CIPARS, *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report*. 2002, Health Canada: Canada. p. 1-89.
8. DANMAP, *Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark*, H.-D. Emborg and O.E. Hueur, Editors. 2002, Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. p. 1-68.
9. Dunlop, R.H., S.A. McEwen, A.H. Meek, R.C. Clarke, W.D. Black, and R.M. Friendship, *Associations among antimicrobial drug treatments and antimicrobial resistance of fecal Escherichia coli of swine on 34 farrow-to-finish farms in Ontario, Canada.* Prev Vet Med, 1998. **34**(4): p. 283-305.
10. Acar, J. and B. Rostel, *Antimicrobial resistance: an overview.* Rev Sci. Tech Off Int Epiz, 2001. **20**(3): p. 797-810.
11. Torrence, M.E., *Activities to address antimicrobial resistance in the United States.* Prev Vet Med, 2001. **51**(1-2): p. 37-49.
12. Interagency Task Force on Antimicrobial Resistance, I., *A Public Health Action Plan to Combat Antimicrobial Resistance*, in *2004 Annual Report*, http://www.cdc.gov/drugresistance/actionplan/, Editor. Centers for Disease Control and Prevention: June 28, 2005.
13. World Health Organization, *The medical impact of the use of antimicrobials in food animals*, in *Report of a WHO meeting*. 1997, WHO: Berlin, Germany.
14. Health Canada, *Uses of Antimicrobials in Food Animals in Canada: Impact on Resistance and Human Health*. 2002, Health Products and Food Branch. p. 1-165.
15. Caprioli, A., L. Busani, J.L. Martel, and R. Helmuth, *Monitoring of antibiotic resistance in bacteria of animal origin: epidemiological and microbiological methodologies.* Int J Antimicrob Agents, 2000. **14**(4): p. 295-301.

16. Statistics Canada, *Table 003-0004[1,2,3] - Livestock surveys, pigs, at end of quarter, quarterly (Head x 1000)*, L.s.-. 3460, Editor. 2004.
17. Dunlop, R.H., S.A. McEwen, A.H. Meek, W.D. Black, R.C. Clarke, and R.M. Friendship, *Individual and group antimicrobial usage rates on 34 farrow-to-finish swine farms in Ontario, Canada.* Prev Vet Med., 1998. **34**: p. 27-264.
18. Dewey, C., B. Cox, B. Straw, and E. Bush, *Use of antimicrobials in swine feeds in the United States.* Swine Health and Production, 1999. **7**(1): p. 19-25.
19. Bush, E.J. and L.G. Biehl. *Use of antibiotics and feed additives in weaned market pigs by U.S. pork producers.* in *Proceedings of American Association of Swine Veterinarians*. 2002. Kansas City, MO.
20. Saenz, Y., M. Zarazaga, M. Lantero, M.J. Gastanares, F. Baquero, and C. Torres, *Antibiotic resistance in Campylobacter strains isolated from animals, foods, and humans in Spain in 1997-1998.* Antimicrob Agents Chemother, 2000. **44**(2): p. 267-71.
21. Payot, S., S. Dridi, M. Laroche, M. Federighi, and C. Magras, *Prevalence and antimicrobial resistance of Campylobacter coli isolated from fattening pigs in France.* Vet Microbiol, 2004. **101**(2): p. 91-9.
22. Aarestrup, F.M., E.M. Nielsen, M. Madsen, and J. Engberg, *Antimicrobial susceptibility patterns of thermophilic Campylobacter spp. from humans, pigs, cattle, and broilers in Denmark.* Antimicrob Agents Chemother, 1997. **41**(10): p. 2244-50.
23. Lucey, B., C. Feurer, P. Greer, P. Moloney, B. Cryan, and S. Fanning, *Antimicrobial resistance profiling and DNA Amplification Fingerprinting (DAF) of thermophilic Campylobacter spp. in human, poultry and porcine samples from the Cork region of Ireland.* J Appl Micro, 2000. **89**(5): p. 727-34.
24. Pezzotti, G., A. Serafin, I. Luzzi, R. Mioni, M. Milan, and R. Perin, *Occurrence and resistance to antibiotics of Campylobacter jejuni and Campylobacter coli in animals and meat in northeastern Italy.* Int J Food Micro, 2003. **82**(3): p. 281-7.
25. Van Looveren, M., G. Daube, L. De Zutter, J.M. Dumont, C. Lammens, M. Wijdooghe, P. Vandamme, M. Jouret, M. Cornelis, and H. Goossens, *Antimicrobial susceptibilities of Campylobacter strains isolated from food animals in Belgium.* J Antimicrob Chemother, 2001. **48**(2): p. 235-40.
26. Moore, J.E. and F. Megraud, *Antimicrobial Resistance*, in *Campylobacters, Helicobacters and Related Organisms*, D.G. Newell, J.M. Ketley, and R.A. Feldman, Editors. 1996, Plenum Press: New York. p. 281-285.
27. Aarestrup, F.M. and J. Engberg, *Antimicrobial resistance of thermophilic Campylobacter.* Vet Res, 2001. **32**(3-4): p. 311-21.
28. PHAC, *Canadian Integrated Surveillance Report Salmonella, Campylobacter, pathogenic E. coli and Shigella, from 1996 to 1999*, in *Canada Communicable Disease Report*. 2003, Public Health Agency of Canada. p. http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/03vol29/29s1/index.html.
29. Guevremont, E., R. Higgins, and S. Quessy, *Characterization of Campylobacter isolates recovered from clinically healthy pigs and from sporadic cases of campylobacteriosis in humans.* J Food Prot, 2004. **67**(2): p. 228-34.
30. Kaneene, J.B. and R. Church Potter, *Epidemiology of Campylobacter spp. in Animals*, in *Microbial Food Safety in Animal Agriculture Current Topics*, M.E.

Torrence and R.E. Isaacson, Editors. 2003, Iowa State Press: Ames, Iowa. p. 175-182.
31. Ewing, W.H., *Serological identification of Salmonella*, in *Edwards and Ewing's Identification of Enterobacteriaceae, 4th ed*, W.H. Ewing, Editor. 1986, Elsevier Science Publishing Co.: New York. p. 201-38.
32. Shipp, C.R. and B. Rowe, *A mechanized microtechnique for Salmonella serotyping*. J Clin. Path., 1980. **33**: p. 595-597.
33. Le Minor, L. and M.Y. Popoff, *Antigenic formulas of the Salmonella serovars. 8th ed.* 2001, Paris, France: WHO Collaborating Centre for Reference and Research on Salmonella.
34. Anderson, E.S. and R.E.O. Williams, *Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology*. J Clin. Path., 1956. **9**: p. 94-114.
35. Ward, L.R., J.D.H. de Sa, and B. Rowe, *A phage-typing scheme for Salmonella enteritiditis*. Epidemiol. Infect., 1987. **99**: p. 291-294.
36. Callow, B.R., *A new phage typing scheme for Salmonella typhimurium*. J Hyg., 1959. **57**: p. 346-59.
37. Anderson, E.S., *The phagetyping of Salmonella other than S. typhi*, in *The world problem of salmonellosis*, E. Van Oye, Editor. 1964, Dr. W. Junk Publishers: The Hague, The Netherlands. p. 89-100.
38. Anderson, E.S., L.R. Ward, M.J. de Saxe, and J.D.H. de Sa, *Bacteriophage-typing designations of Salmonella typhimurium*. J Hyg., 1977. **78**: p. 297-300.
39. Demczuk, W., G. Soule, C. Clark, H.-W. Ackermann, R. Easy, R. Kahkhria, F. Rodgers, and R. Ahmed, *Phage-based typing scheme for Salmonella enterica serovar Heidelberg, a causative agent in food poisonings in Canada*. J Clin. Microbiol., 2003. **41**: p. 4179-4284.
40. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken, *Manual of Clinical Microbiology*. 8th ed. 2003, Washington, D. C.: ASM Press. 666.
41. Clinical and Laboratory Standards Institute, C., *Performance standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*, in *Approved Standards - Second Edition, M31-A2 (Use in Animals)*. p. 55-59.
42. Clinical and Laboratory Standards Institute, C., *Performance Standards for Antimicrobial Susceptibility Testing*, in *Fifteenth Informational Supplement M100-S15 (Use for Humans)*. 2005. p. 102-106.
43. Sorensen, O., M. McFall, S. Rawluk, G. Ollis, M. Schoonderwoerd, and K. Manninen. *Salmonella enterica in Alberta slaughter hogs*. in *International Symposium on the Epidemiology and Control of Salmonella and other foodborne pathogens in pork*. 2001.
44. Rajic, A., M.E. McFall, A.E. Deckert, R. Reid-Smith, K. Manninen, C. Poppe, C.E. Dewey, and S.A. McEwen, *Antimicrobial resistance of Salmonella isolated from finishing swine and the environment of 60 Alberta swine farms*. Vet Microbiol, 2004. **104**(3-4): p. 189-96.
45. CIPARS, *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report*. 2003, Health Canada: Canada. p. 1-110.
46. Gebreyes, W., P. Davies, and P. Turkson, *Characterization of antimicrobial-resistant phenotypes and genotypes among Salmonella enterica recovered from*

pigs on farms, from transport trucks, and from pigs after slaughter. J Food Prot , 2004. **67**(4): p. 698-705.

47. Farrington, L.A., R.B. Harvey, S.A. Buckley, R.E. Droleskey, D.J. Nisbet, and P.D. Inskip, *Prevalence of antimicrobial resistance in Salmonellae isolated from market-age swine.* J Food Prot, 2001. **64**(10): p. 1496-502.
48. Gebreyes, W.A., S. Thakur, P.R. Davies, J.A. Funk, and C. Altier, *Trends in antimicrobial resistance, phage types and integrons among Salmonella serotypes from pigs, 1997-2000.* J Antimicrob Chemother, 2004. **53**(6): p. 997-1003.
49. NARMS, *Escherichia coli - 2000 Report*, http://www.ars.usda.gov/Business/docs.htm?docid=6770, Editor. 2000, United States Department of Agriculutre: July 5, 2005.
50. Teshager, T., I.A. Herrero, M.C. Porrero, J. Garde, M.A. Moreno, and L. Dominguez, *Surveillance of antimicrobial resistance in Escherichia coli strains isolated from pigs at Spanish slaughterhouses.* Int J Antimicrob Agents, 2000. **15**: p. 137-142.
51. Dunlop, R.H., S.A. McEwen, A.H. Meek, R.C. Clarke, W.D. Black, and R.M. Friendship, *Associations among antimicrobial drug treatments and antimicrobial resistance of fecal Escherichia coli of swine on 34 farrow-to-finish farms in Ontario, Canada.* Prev Vet Med, 1998. **34**(4): p. 283-305.